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Bang!—you're seriously injured

WHAT constitutes inhumane weaponry? Some, doubtless dismissed as soft-headed do-gooding pacifists, might wonder whether humanity can solve any dispute by projecting a small metal object at a velocity of up to 1 kilometre per second into the body of an adversary causing all manner of totally unpredictable damage. But so sated are we, as a culture, with letting the disagreements of individuals, groups and nations be settled by technological means totally unrelated to the merits and demerits of the case in question that the issue of just what weapons a nation can acceptably deploy has had to be faced for more than a century, and will be the subject of a United Nations conference later this year.

The St Petersburg Declaration of 1868 was the first international agreement on the subject, and its principles are worth repeating even today. They state that the only legitimate objective should be to weaken the military forces of the enemy, and for this purpose it is sufficient "to disable the greatest possible number of men". They go on to state that it would be contrary to the laws of humanity to exceed this objective by employing arms which "uselessly aggravate the sufferings of disabled men or render their death inevitable". The principles that military actions should not be indiscriminate, particularly avoiding civilians, and that weapons which cause injuries having no military utility should be banned, have continued to be at the centre of conferences ever since. In 1907 (in response to the threat of chemical warfare) the concept was added at the Second Hague Peace Conference that cruel and repulsive weapons, even with military utility, should also be prohibited. Needless to say these ideals have been widely ignored.

SIPRI, the Stockholm International Peace Research Institute, today publishes a valuable guide to the subject of inhumane warfare. *Anti-personnel Weapons* (Taylor & Francis, £9.00) by Dr Malvern Lumsden, a researcher at SIPRI, is an attempt to bring together all the scattered information available about ammunition, grenades, bombs and mines used primarily against people rather than matériel. This is done within a historical framework, making it particularly easy to see the evolution of weaponry and attitudes. Dr Lumsden also includes a brief discussion on emerging methods such as acoustic and laser techniques.

The St Petersburg Declaration was in part a response to a general concern in the mid-nineteenth century that exploding bullets might come into general use, but already by then conical bullets were inflicting more severe wounds than older spherical ammunition, and it was beginning to be noted that bullets could be designed to splay out on encountering an obstacle. Eventually, of course, this led to the Dum-Dum bullet, having a soft metal nose which expanded on penetration, causing extensive internal damage. Britain argued for the Dum-Dum bullet in colonial operations at the Hague conference of 1899, saying that whilst soldiers were incapacitated by a single projectile, "the savage, although run through two or three times, does not cease to advance"; hence the need for a more damaging bullet. But the conference reaffirmed the need to avoid superfluous injuries.

Twentieth century warfare has been somewhat different. Certainly bullets have continued to be widely used and many of these, fired from lighter weight rifles, have been of smaller calibre. This diminishes effectiveness if the bullet just drills a narrow hole through the body, but if it can be made to tumble and even fragment within the body, damage levels can be greatly raised. All current bullets are capable of causing this damage, which is reminiscent of that from the explosive bullets of the 1860s. But rifle and machine gun fire has been less successful during the First World War and subsequently at disabling the enemy so much as at containing him; up to fifty thousands rounds may be expended in modern warfare for every hit made.

In the meantime, fragmentation weapons have grown in importance until now they account for 70% of all casualties. Recent trends in bombs, grenades and shells have been to design delivery systems which disperse large numbers of small fragmentation weapons over a wide area, and to increase the number of fragments produced from individual explosions. Furthermore, weapons can be made to burst just above the ground, say at eye level.

Fragmentation weapons raise questions concerning indiscriminate use. Some can be delivered accurately but many are sufficiently widely scattered that there can be no guarantee that civilians are not equally victims of them. Delayed-action devices such as mines fall into the same indiscriminate category, often being triggered months or even years later by a non-combatant. It would be possible, however, to incorporate a neutralising device in a mine to put it out of action after a given time.

Dr Lumsden reports on these and many other devices which have been used or at least investigated, such as fuel-air explosives, concussion bombs, flechettes, toxic bullets, baton rounds, rubber bullets and so on. He describes them all dispassionately, in the best SIPRI tradition, and the book deserves to be read and thought about by a wide range of people who would not normally give serious attention to conventional warfare.

What, then, are the general questions confronting international discussions? First one must ask whether it is really possible to walk at all on the knife-edge between acceptable and unacceptable warfare. Can a line be drawn between serious injury from a tumbling bullet and death from a non-tumbling one? And can civilians and military forces really be distinguished in modern guerilla warfare? As Lumsden points out some of the problems of fair and foul fighting were faced up to during the United States' involvement in Indo-China, and the Rules of Engagement at that time deserve serious study as a basis of a way forward. Second, what is it in us that regards chemical weapons as crueller even than nuclear weapons and much crueller than guns and bombs—and is there any possibility that public opinion could in future be made to regard nuclear and conventional warfare as equally cruel? Third, is it best that we convene technical and medical conferences for precise definitions of prohibitions, a small step forward for arms control, or put all our effort into more extensive but more inaccessible measures of disarmament? □



US scientists to join Italians in dioxin study

An announcement is expected later this month of a joint scientific investigation into the health implications of exposure to dioxin. **Alastair Hay** reports on the protracted negotiations leading to this decision.

In February the US National Academy of Sciences (NAS) will announce the formation of a binational study group to consider the public health consequences of exposure to 2,3,7,8-tetrachlorodibenzodioxin (dioxin). Behind the decision to form the group—composed of equal numbers of Italian and American scientists—lies a history of nearly two years of discussion, negotiation and some doubt as to the value of what was seen by some as nothing more than yet another committee looking into the dioxin problem.

The initiative to form the study group was taken by the NAS shortly after the accident at Séveso in Italy in July 1976 when dioxin from a trichlorophenol reactor was discharged over a populated area. In the words of one prominent Italian scientist, the NAS wrote to the National Research Council in Italy recommending a study of Séveso. The suggestion was couched in terms of the NAS "offering help to Italy".

It seems however, that, at that time, Italian scientists were not over-eager to conduct the study proposed by the NAS. The letter which the NAS sent went unanswered until a senior Italian scientist happened to see it by accident. Recognising its potential importance he brought it to the attention of the Minister of Health urging him to reply favourably and to invite the NAS to send a team to discuss the proposition.

The team, led by Norton Nelson, Professor of Toxicology at New York University, visited Italy in early April 1977.

When it returned, it made several recommendations including suggestions for developing a continuing relationship between US scientists and their Italian counterparts; the exchange of scientific and technical literature; the initiation of complementary research; and the organisation of workshops and exchange visits. It was suggested that the US programme be operated under a small steering group appointed by the NAS and its own National Research Council and that it relate to an 'appropriate counterpart' committee on the Italian side.

These recommendations were subsequently sent to Italy for approval together with a request for the names of the Italian scientists who would be participating in the programme. According to the NAS the matter then

'lay dormant' until late January 1978 when a reply was received from Dr Pocchiari, Director of the Istituto Superiori Di Sanita in Rome, naming the Italian members and requesting the formation of the NAS counterpart committee.

It seems to have been some months before the NAS prepared its final programme for which it sought funding. The project it proposed was to be conducted in several phases, the first being simply a review of the situation. Subsequently the study groups activities might include "design and evaluation of medical follow-up studies, methods, and design of systems for emergency response to similar accidents". Thus the NAS made it quite clear that it would consider a range of issues stretching beyond Séveso. Indeed, in its proposal the NAS Assembly of Life Sciences said that it would try to "develop guidelines and approaches to handling of future tragedies, and to the forecasting of potential environmental disasters".

Norton Nelson, who will be one of the US members of the study group, qualified the last proposal by saying that he is always wary of "cook-book recipes for accident prevention in future" and that he would be content if the joint programme merely provided some general guidelines for action.

With no funds of its own to implement the joint study programme, the NAS sought support from several other sources. According to an NAS spokesman the programme—which will last initially for two years—has been funded with some \$120,000 by the Environmental Protection Agency, National Institute for Environmental Health Sciences and the Centre for Disease Control. Asked if F. Hoffman la Roche—the parent company which owns the reactor at Séveso—had been approached for funds as well the spokesman insisted that it had not. One of the US study group members added that Roche would be "amongst the last we would approach" for support.

The National Academy, however, did consider asking Roche for funds, early in 1978. When informed about the NAS's suggestion to approach Roche the Italian government made it quite clear that it would have no part in the study if the company played any part in funding it. Embarrassed at having considered such an approach

and anxious to avoid the cancellation of its programme the NAS had to seek its finance from alternative sources.

The NAS committee members have "not yet met", according to Nelson, but will do so in early February. Following this meeting the US scientists will then meet with their Italian counterparts. "Out of that meeting we hope to formulate a programme of action", said Nelson.

Sorting out that programme could be a delicate exercise. If, as one Italian scientist put it, "it is just a NAS study in Italy, then that is very difficult and complicated. On the other hand, an international study is fine". What the Italians are looking for is a clear definition of the terms of reference of the NAS study.

One of the proposals of the NAS is that an epidemiology panel be set up. But one senior Italian scientist said that although the health surveillance programme of the Séveso residents conducted by the Lombardy Regional Council had been less than successful he was "not sure that a new epidemiological study would work".

The protracted nature of the negotiations to establish the study group has left one of the Italian group members less than enthusiastic about the proposals, particularly as the situation at Séveso has changed so much. One other criticism of the binational study group, advanced by some scientists, is that it is yet another committee set up to consider dioxin. Italian scientists say that the International Agency of Cancer (IARC) was to coordinate all information on dioxin yet that they have heard no more from the IARC.

Nelson denies that there will be any "duplication of effort". He says that both the IARC and World Health Organisation know about the NAS proposals and will be kept thoroughly informed.

Of one of the anxieties expressed by Italian scientists that the NAS programme could be read as a criticism of their own activities at Séveso, Nelson said that this should not be the case. He felt that after the two week delay in evacuating the residents of Séveso, the authorities had acted swiftly. The NAS said Nelson had "no complaints about the handling of the situation by the Italians".

The binational study group has recently been described by one US scientist as a "committee of good intentions". Many other US scientists hope that it will be more than this and they seem relieved that the programme has actually got off the ground at last.

□

Hope for UNCSTD in New York

David Dickson reports from New York on the third meeting of the preparatory committee for the United Nations Conference on science and technology for development

WITH less than seven months to go until the United Nations Conference on Science and Technology for Development (UNCSTD) opens in Vienna the political pace of preparations is beginning to heat up with indications that the conference may not turn out quite so unproductive as some have been fearing.

The most positive indication, according to a number of delegates attending the third meeting of the conference's preparatory committee, which opened in New York last week, is a new draft preliminary programme of action prepared by the conference secretariat.

The new draft, although still felt by many to suffer a number of important omissions—such as adequate discussion of the role of women in development, or the need for appropriate technologies—is widely accepted as a major improvement on earlier versions, which had been little more than the compilation of suggestions made in national and regional papers.

In particular, the new draft contains—as the General Assembly of the United Nations has said that it should—a number of specific recommendations for action around which discussion is likely to focus in the months ahead.

Opening the committee meeting last week, Dr Joao Frank da Costa, Secretary-General of the conference said that it was often more difficult to

elaborate principles than to elaborate specific action-oriented measures, and that he therefore suggested concentrating on the latter rather than the former.

In particular, Dr da Costa said it would be necessary to develop firmer guidelines on cooperation between developed and developing countries, while the developing countries themselves, for which UNCSTD was a “now or never opportunity”, should investigate ways of increasing their collective self-reliance.

The preliminary draft programme of action, which will be revised following comments by delegates at the New York meeting, contains both a theoretical and conceptual framework for further actions based largely on the outcome of debates, within the UN system, on the shape of the new international economic order, as well as over one hundred concrete measures for action at all levels.

Most of the recommendations are relatively uncontroversial. They include the suggestion that the developed countries increase the amount of money they spend on research into the problems of developing countries, and that the latter should establish national science and technology policies—drawn up and executed by governments, though with the advice of broadly based national science and technology councils representing all sectors of

society—and establish institutions for assessing the impact of transferred technology.

More likely to generate controversy are recommendations which would involve setting up new international funds. The draft, for example, suggests that developing countries should establish a common science and technology fund “to which the most prosperous among them should contribute more generously”, and that consideration also be given to a “risk capital fund” associated with existing financial institutions within the UN system.

Equally controversial are suggestions that to go beyond the domain of science and technology to comment on more overtly political issues. Paragraph 11.12, for example, suggests that in parallel to mobilising science and technology for development, “countries should introduce internal structural policies such as social reforms, redistribution of income and so on, as appropriate, so as to ensure that the application of science and technology does not result in social tensions”.

How many such suggestions will survive the revision process remains to be seen. Of particular interest is whether the developing countries, through the group of 77, will be able to agree on a common set of demands. A working party established last week by the group is said to have had initial difficulty reaching a consensus. But the more coherent their position, according to observers in New York, the more likely that controversial issues will stay on the UNCSTD agenda. □

US paper stresses modernisation and meeting basic human needs

THE modernisation of developing economies, and meeting the basic human needs of developing country populations are the two over-riding objectives in applying science and technology to development, according to the US national paper for UNCSTD.

In the introduction to the paper, Father Theodore Hesburgh, previously president of Notre Dame University and chairman of the US delegation to the conference, says that these two goals are not mutually exclusive or incompatible. “The fulfilment of basic needs and the process of economic moderation can proceed simultaneously in a mutually enforcing relationship,” says Father Hesburgh.

The US paper, which has gone through numerous drafts and is yet to be officially released, describes the role played by science and technology in the growth of the US economy, as well as the US's experience in applying

scientific and technological expertise to the problems of development in other nations.

The paper points out that President Carter has made scientific and technological cooperation a key element of the US's relationship with other countries, particularly in the developing world. It says that the success of the developmental process in other nations is “very much in the US interests.”

The paper lists recommendations for action in six areas:

- Improving the capacity of industrialised countries to assist developing countries upgrade their scientific and technological skills;
- Assisting the development of education and training programmes;
- Encouraging collaborative research between scientists and engineers from both developed and developing nations on global problems such as food, water and health;

- Helping to overcome obstacles to developing countries' access to scientific and technological information;
- Strengthening the links between the research communities of developed and developing nations, including joint research and problem solving;
- Building a more effective UN system for science and technology.

The US paper puts forward the Foundation for International Technological Cooperation, which has been proposed by President Carter, as one way in which scientists from the developed and developing nations could work together, and suggests that the UN conference “should encourage the establishment of similar institutions in other countries”.

The paper also says that the many useful science and technology activities within the UN system need a central focal point and policy direction, although, it adds, not a new entity. □

Brazil bans conference on Amazon biology

THE Brazilian government has refused permission for a conference on the evolution of biological systems in the Amazon region, organised by the Association for Tropical Biology, which was to have opened next week in Manaus, the region's major city.

Although no official reason for the decision has been given, scientists in the US believe that the military government may have been concerned that those attending the meeting would use it as an occasion to criticise the environmental effects of Brazil's current development policies—even though the conference itself will deal primarily with events that took place over 5,000 years ago.

The conference has been hastily rearranged to take place in Macuto, on the Venezuelan coast outside Caracas, and has received the support of the Venezuelan Ministry of the Environment. It has been convened to discuss biological models of diversification in the tropics. Speakers will address topics



The Amazon: science can help protect its environment

such as the relationship between climatic change and species differentiation in the Amazon and its surrounding savannah region.

All the original speakers—including a number from Brazil—are expected to attend as planned. However, the change of location may prevent the participation of some Brazilian research workers and students, for whom lengthy visa negotiations, as well as the deposit of a large sum of money, are necessary for foreign private travel.

"This conference will bring together specialists in disciplines ranging from archaeology to entomology and genetics. Everything is now working together, and we are coming up with some exciting material, such as the

correlation between archaeological sites and past climatic changes," Dr Clifford Evans, curator of South American archaeology at the Smithsonian Institution in Washington, DC, and one of the main organisers of the conference, said last week.

The military government's decision, which was taken on the advice of the National Security Council, has provoked a storm of protest in the Brazilian press, from which censorship has only recently been lifted. Critics, including a number of eminent scientists, have accused the government of interfering with academic freedom, and of ignoring how science is necessary to preserve the environmental integrity of the Amazon region.

David Dickson

Second thoughts on study of nuclear risk

THE Boston-based Union of Concerned Scientists has called for the temporary shutdown of 16 US nuclear power plants for safety modifications, in the light of evidence that previous estimates of "low probability" risks may have been unjustified.

This demand follows the decision of the Nuclear Regulatory Commission to withdraw its support from important parts of a study published by the commission three years ago on the safety of nuclear reactors, which indicated that the risks from nuclear power were much less than from other man-made activities.

The study was carried out for the NRC by a team headed by Professor Norman Rasmussen, of the Massachusetts Institute of Technology. On its publication in 1975, the NRC described the report as a "soundly based and impressive work".

The conclusions of what has become widely known as the Rasmussen Report have been frequently used by supporters of nuclear power to defend existing and proposed programmes. However, criticism by others of the report's methodology and its conclusions—in particular by Congressman Morris Udall, chairman of the House Committee on Interior and Insular Affairs—led the NRC to appoint a

review group headed by Professor Harold Lewis of the University of California, Santa Barbara.

In its report, which was presented to the commission in September, the review groups said that it found the methodology used in the study—based on a fault-tree/event-tree form of analysis—to be basically sound. However it had a number of reservations about the way that the results of the analysis had been presented.

In particular, the committee criticised the narrow limits which the Rasmussen report had placed on estimates of the probability of a melt-down of the reactor core. The reasons for understating the error band, said the committee, included an inadequate data base, a poor statistical treatment, and an inconsistent propagation of uncertainties throughout the calculation.

The review committee also criticised the executive summary of the report—the part which has been most widely quoted in public discussions. The committee says that the summary did not sufficiently emphasise the uncertainties involved in calculating the probability of risk and that this might have led readers to a "misplaced confidence" in the validity of the risk estimated.

In a statement issued last week, the NRC commissioners say that they

accept the criticisms of the review committee, and as a result have withdrawn any explicit or implicit endorsement of the report's executive summary.

On accident probabilities, the NRC says that the absolute values of risk in the Rasmussen report "should not be used uncritically either in the regulatory process or for public policy purposes". (The report states, for example, that nuclear power plants were about 10,000 times less likely to produce fatal accidents than man-made non-nuclear activities.)

The NRC's decision to support the Lewis Review Committee's findings has prompted the 65,000 strong Union of Concerned Scientists, which has been among Rasmussen's most vocal and persistent critics, to demand additional safety precautions at 16 plants where, they claim, there is evidence that the report was used to justify the acceptance of current safety procedures.

"Although the risks are still indeterminate, the uncertainty band includes an area of risk which is unacceptable, and it is on this basis that we are asking for the shutdowns", Dr Henry Kendall, Professor of Physics at Massachusetts Institute of Technology, said last week.

David Dickson

HSE confident it can cope

THE UK Health and Safety Executive (HSE) is confident that it is adequately staffed to inspect all UK laboratories doing genetic engineering experiments.

In reply to questions by the House of Commons Select Committee on Science and Technology last week, the HSE said that its three specialist inspectors for microbiological research could cope with inspecting the UK's seven Category Three and two Category Four laboratories. The time lag between notification of a laboratory's intent to conduct Category Three or Four experiments and inspection had never exceeded six weeks.

Laboratories conducting Category One and Two experiments do not need to be inspected before work can commence. The HSE felt that because the inspection of these laboratories does not require such specialist knowledge, regional inspectors of medical laboratories could cope with them. So far, about 200 experiments in 50 centres have been notified to the Genetic Manipulation Advisory Group (GMAG). Since last July the HSE and GMAG have jointly inspected 14 of the centres.

GMAG has recently had a change of membership. Seven new members, J. Chamberlain, Sir Frederick Dainton, J. Ingle, M. Kogan, C. M. Puxon, P. M. B. Walker and P. Wildy together with a new chairman Sir William Henderson started work in January. Under its new chairmanship, GMAG may be taking a more positive attitude to work with recombinant DNA. Sir William has few fears about the potential scale-up of the processes in industry. He told the select committee at a previous meeting that "the hazards of scale-up are very much less than has been anticipated". He believes that 4,500 litres of reagent in clean, sealed stainless steel flasks are probably safer than the intermediate stage: "the danger is when you move from the bench to the 5 or 10 litre scale, when you don't have adequate procedures."

GMAG is setting up a subcommittee on scale-up, and Sir William is playing close attention to its membership. He feels it should have a strong representation of chemical engineers who have experience of risk assessment in industry.

Dr Ken Duncan of the HSE shares Sir William's lack of fear about the dangers of scale-up to industrial processes when this happens, he says, the HSE will be able to call on its chemical engineers, currently engaged in other activities within the HSE, for advice. Duncan, however, does not foresee unusual problems with scale-up. □

UK tightens up on lab safety

MANY OF the recommendations of the Shooter report, which called for tighter laboratory safety regulations following the smallpox outbreak at Birmingham University, are now to be implemented by the Government. The move follows the unofficial "leaking" of the report by Clive Jenkins, general secretary of the Association of Scientific, Technical and Managerial Staffs. The Government's decision reveals that it no longer considers the country's voluntary safeguards for handling viruses in laboratories as adequate.

Social Services Secretary David Ennals told the House of Commons last week that several changes were to be made. These include:

- Regulations compelling laboratories to give notice of their proposed work.
- An official licensing system to replace the present voluntary method of approving category A pathogen laboratories.
- Accepting the recommendation that category A laboratories should be inspected annually.
- Setting-up a review of the Dangerous Pathogens Advisory Group, the body responsible for approving these laboratories, to find ways of broadening its membership to include wider union and public interest.

No new legislation will be introduced to carry out these measures, which will be implemented as part of the Health and Safety at Work Act. Mr Ennals said public fears following the smallpox outbreak that killed medical photographer Janet Parker last September should not be allowed to cause panic. If overprotective measures were introduced, there could be great damage to the ability to carry

out effective diagnosis or research.

Mr Ennals also revealed that a re-inspection of existing category A pathogen laboratories—which handle smallpox, rabies, lassa fever and other dangerous viruses—is now being carried out by the Health and Safety Executive. "In some cases it has become clear that improvements in safety procedures are necessary and immediate action has been taken", he said.

Details of these cases of "immediate action" were revealed by the HSE last week and consisted of stopping work with category A pathogens at two laboratories. The first, the public health laboratory at Colindale, North London, the only centre for rabies diagnosis in Britain, was visited on January 18 by HSE inspectors and a request for an end to dangerous virus work was issued.

The order was made in the form of a Crown notice, which is not legally enforceable as one Government body cannot prosecute another, although the HSE has recently called Mr Ennals to remove Crown immunity for such laboratories. However, the director of Colindale, Dr Eric Mitchell, said a new purpose-built virus laboratory was to be opened there in a few weeks—after it had been inspected by the HSE, DPAG and the Home Office.

The other laboratory, at the Lister Institution in Elstree, Hertfordshire, has been asked to improve safety precautions in the production of rabies vaccines. The laboratory was closed by the institution last June, and the HSE has instructed that it must not re-open without its approval.

Robin McKie

Switzerland to consider revised guidelines

Two months ago Mrs Shirley Williams, Secretary of State for Education and Science speaking on the television programme *Weekend World*, said that she would like to see the establishment of some form of international guidelines to regulate research on recombinant DNA. Commenting on the different guidelines already in operation in the United States, Britain and the European Community, Mrs Williams singled out Switzerland as one country operating outside these formal rules. There was a need, she said, to induce it to come within some form of "containment barrier".

Switzerland is of course the home of the large multinational pharmaceutical companies F. Hoffmann-La Roche, Sandoz and Ciba-Geigy. Roche and Ciba are both aware of the com-

mercial possibilities of genetic engineering—and both have a finger in the pie. Roche sponsors the Basle Institute for Immunology and Ciba the Friedrich Miescher Institute, also in Basle. Both institutes have begun recombinant DNA research which is, as yet of a fundamental, rather than an applied nature.

But all recombinant DNA research in Switzerland whether commercially, privately or publicly sponsored is subject to certain controls. The controlling agency is a commission set up by the Swiss Academy of Medical Sciences in 1975. In an open letter dated April 1978, the commission set out its control policy. It was the commission's view that the U.S. National Institutes of Health (NIH) guidelines should operate in Switzerland and it recom-

mended this to all practitioners in the field. The commission also made it clear that the responsibility for the safety of this work lies with the principal investigator.

Professor Werner Arber of the Biozentrum at the University of Basle, and one of the 1978 Nobel Laureates in Medicine is a member of the commission. Commenting on the responsibility of the investigator, he told *Nature* that: "It is perhaps important to say that the Swiss Law of Epidemics foresees that any work with pathogens and their products has to be carried out with the maximum of care, and our commission interprets this formulation in such a way that recombinant DNA [research] done in our country has also to be done with a maximum of care".

The commission compiles an annual register of all work done in genetic engineering. In 1978 there were some 31 projects in the field under 18 principal investigators, eight of whom were based in Basle. The projects are divided into two groups according to their source of DNA for recombination. Category 'a' experiments are those using cellular DNA and using *E. coli* as host; and category 'b' includes those using DNA from plasmids, bacteriophage and viruses and again using *E. coli* as the host. Twenty-two of the projects are in category 'a' and include work with embryological tissue from primates, other mammals, birds and cold blooded vertebrates. Eight of the 'a' category are listed as using 'inferior eukaryotes' as a source of DNA. In category 'b' the recombination work is listed as involving plant viruses, 99% pure organelle DNA from eukaryotes (but not primates) and plasmids or phage DNA from hosts, the genes of which are interchangeable with *E. coli*.

Of the 18 principal investigators 15 are based in universities, with the remaining three privately sponsored.

It is the European Science Foundation (ESF) which is trying to harmonise the guidelines and working conditions in recombinant DNA research in Europe. Arber represents the Swiss Commission at the ESF meetings and appreciates the effort the Foundation is making to find the common ground. However, Arber says that the Swiss Commission has still not reached a firm decision on the guidelines it will adopt in the future. In the next few months the commission will meet again to consider the revised NIH guidelines (which lift restrictions on some categories of recombinant DNA research, but which for the first time extend the controls to cover industry), those of the UK Genetic Manipulation Advisory Group, and the guidelines proposed for France and Germany.

Alastair Hay

Catch a falling Kosmos—and send the bill to Moscow

OPERATION Morninglight—the clean-up of northern Canada, following the "unscheduled re-entry" and crash of the Soviet satellite Kosmos-954 a year ago is now over, at a cost of some \$13 million. Canada is sending a bill for \$6 million to the Soviet Union.

Kosmos-954, which carried a power-source fuelled with some 50 kg of enriched uranium, crashed in the area of the Great Slave Lake on 24 January 1978. The first stage of Morninglight was launched within hours—a massive air search for radiation hot-spots. Within four days, three radiation hot-spots were located, and several more within the next week.

A party of six meteorologists encamped in the Warden's Grove area, two of whom actually touched a fallen fragment, were evacuated to Edmonton for radiation tests; but they showed no signs of contamination. As for the local Chipewyan Indian population, apart from a missing pair of trappers who were feared to be camping near a hot-spot, the greatest danger seemed to be fear and bewilderment as to the nature of the search, plus the ominous appearance, in the settlement at Snowdrift, of a wolf which refused to be driven away and finally had to be shot.

The second stage of Morninglight—a ground level survey—was scheduled for July, when the snows had melted. Shortly before it was to begin, uranium prospectors reported satellite debris in northern Saskatchewan, and the search was extended beyond the Great Slave Lake area. Morninglight finally covered some 90,000 square kilometres, from which over 3,000 pieces were recovered, mostly small particles, but with a few larger fragments, one of which was said to be—"as big as a five gallon drum". One of the larger pieces was so radioactive that a special lead container had to be built by the University of Alberta. Another fragment, though, a 225 cm x 50 cm stove-pipe shaped cylinder, was sufficiently "cool" to be loaned for temporary exhibition to the National Museum of Science and Technology in Ottawa.

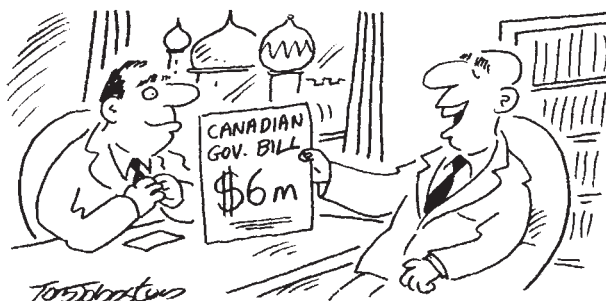
By the beginning of winter, when phase two of Morninglight ended, the Atomic Energy Board of Canada announced that the residual radiation risk in the impact area was minimal. Any remaining radioactive debris would soon decay to below natural background level, and the hazard to humans or wild life from accidentally ingested particles would be no greater than that from a medical x-ray examination. Nevertheless, as an additional precaution, the Federal Environment Department would monitor fish from the Great Slave Lake, while the Health Department would check the meat of migrating caribou herds.

Apart from these minor hazards, and the \$13 million bill, what are the lasting effects of the Kosmos-954 incident? First, the setting up, on Canada's initiative, of a UN working group to study the technical aspects and safeguards for nuclear power sources in space, the first meeting of which will take place later this year. Minor spin-offs include a considerable amount of practice in the aerial and ground location of radioactive debris, and the (at least temporary) appearance on the map of the name "Cosmos Lake" to denote the base camp of the aerial search.

Canada's claim against the Soviet Union will doubtless provide international lawyers with fascinating material on compensation norms and procedures. As far as the average Canadian is concerned, there remains a profound sense of thankfulness that the impact took place in a virtually uninhabited area—and considerable apprehension of what could happen next time.

According to NASA officials, there is a 2% chance of debris from Skylab striking Canada. Moreover any such impact would occur south of latitude 50°N—a band which includes all major Canadian cities except Edmonton and Calgary. Not surprisingly, Canadians are said to find the news of the forthcoming descent of Skylab to be, at the mildest, "somewhat disturbing".

Vera Rich



"There's a fair chance that the Skylab crash will wipe out all record of this bill, comrade!"

news in brief

ASTMS branch wins redundancy rights: A local branch of the Association of Scientific Technical and Managerial Staffs has persuaded the Royal Free Hospital School of Medicine to withdraw waiver clauses concerning redundancy rights from its fixed term contracts. The school secretary, Mr G. W. Fenn, refused the initial ASTMS request. Negotiations were then referred to the school's joint negotiating committee, composed of three ASTMS representatives and three administration representatives. They also rejected the proposal but after discussion agreed to present the request to the full school council (from which ASTMS representatives are excluded). The council agreed to withdraw the part of the waiver clause requiring employees on fixed term contracts to give up their rights to redundancy pay but have retained the waiver for rights against unfair dismissal. "To maintain happy relations in a small organisation like ours, we felt it was a good thing to do for the employees," said Fenn.

UNEP calls key meeting on Mediterranean pollution: The United Nations Environmental Programme is convening a meeting of the Mediterranean Action Programme from 5-10 February to discuss the activities of the 1979-1980 MAP programme, the location of MAP headquarters and most important, the share out of \$2½ million operating expenses among the 17 member states. Some struggle is expected about the exact terms of the share out but the conference is expected to make slow steady progress towards the eventual goal of a full Mediterranean clean-up. The major problem, towards which the conference will make a small contribution, is land-based pollution—industrial water, domestic sewage, agricultural pesticides and fertilisers—which account for 85% of Mediterranean pollution. The World Health Organisation estimates that it will cost \$5 billion over a period of 10 to 20 years to control pollution at the source, often hundreds of kilometres upstream from the Mediterranean.

US prepares for Skylab re-entry: The US government has set up an interagency group to prepare for possible damage when Skylab re-enters the earth's atmosphere later this year. Dr Robert Frosch, administrator of the National Aeronautics and Space Administration, told a Senate panel last week. The State Department, the Department of Defense and the New Federal Emergency Management Agency are working with NASA to set up an international warning system and an assistance team to deal with any damage or injury caused by debris from the 80-ton space station as it re-enters the atmosphere.

Dr Frosch told the Senate subcommittee on space and science: "There are potential options of exerting some influence on what happens to Skylab during its last couple of orbits." But, he said, NASA scientists did not yet know how much control they would have over Skylab's break-up—and in particular whether they could bring it down over an ocean. One critical factor was the power supply, and whether Skylab's solar panels could be kept fully charged. If Skylab could not be reliably brought down into the sea, it might be wise not to try to influence its descent, Dr Frosch suggested, because of the political implication of the US trying to crash the debris into a particular part of the globe.

Sullom Voe oil spill: Census taken by the Royal Society for the Protection of Birds after the Shetland Island oil spill, where 1,150 tonnes of heavy bunker fuel oil fouled fifteen miles of coastline has shown the spill to be "locally disastrous" according to Bobby Tulloch, RSPB representative. 2,800 birds from 45 species have been killed. Of the local

eider population of 350 birds, 5 are left, with a similar picture extending to other species. Twenty others have been killed or "badly affected" and it is not clear whether any remain. Local sheep that graze on seaweed deposited along the rocky coast have become polluted with unknown toxic and carcinogenic effects.



Soviet award for Todd: The Lomonosov medals, the highest awards of the Soviet Academy of Sciences, are awarded each year to one Soviet and one foreign scientist. The 1978 awards, announced last week, go to Academician Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, for his work in nuclear physics, and Lord Todd (left), president of the Royal Society, for his work in biology. The linking of the two Presidents in this way is something of an auspicious coincidence. The Royal Society has a long standing scientific exchange programme with the Soviet Academy, dating back to 1956 and the very beginning of the post-Stalin "thaw". The then Dr Alexander Todd helped establish this exchange.

British students swing to science: Statistics from the two major school examination boards in Britain are showing a trend back towards physical sciences at A-level, at the expense of subjects like history and geography. Recovery from the slump in interest of the mid-1970s is particularly apparent in chemistry, where the number of candidates entering the London Schools Examination Board A-level has increased from 14% to 16% in the last four years, while the Joint Matriculation Board's chemistry candidates accounted for 21.3% of total entries, the highest proportion since their comparative statistics began in 1971. Physics and mathematics are following the same trend, with increases of around 1% to 2% over the past two to three years. With a total A-level entry to these boards of 66,000 and 55,000 respectively in 1978, even a small percentage increase indicates a significant swing towards science.

However, Professor L. A. B. Elton of the Institute for Educational Technology, Surrey University, considers the statistics as presented are "too bland for anyone to draw sensible conclusions". They do not distinguish between male and female candidates, for example.

Seasat failure due to technical and management faults: The failure of the Seasat satellite, which was launched early last year by NASA and which ceased transmitting data in October, was the result of a "massive and progressive" short circuit, due to a "lack of proper attention" by NASA's jet propulsion laboratory and the Lockheed Missile and Space Company to testing of the rocket stage to which the satellite was attached. "As a result, a test was waived without proper approval, important component failures were not reported to project management, and flight controllers were inadequately prepared for their task," said a board of enquiry in a report issued last week.

Seasat was launched to test equipment for gathering data on the world's oceans for both scientific and meteorological purposes. Before the failure occurred the satellite had been transmitting data for 99 days. The report says that the most likely cause was an electrical arc between adjacent slip ring brush assemblies.

Correction: President Carter has requested a budget increase for basic science of 2% above inflation, not 20% as reported on page 252 last week. . . . alas.

Does the UK have a numeracy problem?

Joseph Schwartz puts his own view of the controversy over the mathematical abilities of UK schoolchildren

IN March 1978 Dame Kathleen Olle-
renshaw, President of the Institute of
Mathematics, released her account of
innumeracy in Great Britain to the
press. "Simple sums floor vast majority
of pupils" was the *Times* headline. An
examination administered to 7,000
15-year-olds in six education districts
produced "abysmal" results.

The test consisted of 17 questions,
of which nine had two parts and two
had three parts, requiring a total of 44
basic operations to be performed in 40
minutes. The problems ranged from 12
computations of the type $14+35=?$ to
nine word problems (How much do I
save if I buy two large bottles of sham-
poo (150 cc @ 25p) instead of taking the
same quantity in small bottles (75 cc
@ 15p)?) and included questions in-
volving the reading of graphs and train
timetables.

Calling a mark of 90% adequate and
a score of 80% well below an accept-
able standard, Institute members were
disappointed in the actual results. The
percentage of pupils receiving an 80%
score in the six districts ranged from
21% to 61%. The percentage of per-
fect papers ranged from 1% to 13%.
Dame Kathleen said "education as a
whole, and parents and employers will
agree, is not doing a good enough
job".

More recently, in September 1978, the
Department of Education and Science
announced the formation of a Commit-
tee of Inquiry into the teaching of
mathematics in schools. This new com-
mittee is a consequence of the 1976-
1977 Tenth Report of the Expenditure
Committee on the Attainments of the
School Leaver in which mathematics
was particularly singled out for atten-
tion. "We heard more statements
about mathematical competence or
lack of it than about any other school
subject. The CBI described it as the
issue most frequently mentioned by
their members".

Dr H R Pitt, a mathematician and
now Vice-Chancellor of Reading Uni-
versity, testified "I think there is no
doubt that there is something of a
crisis in the teaching of mathematics",
attributing the crisis to the introduction
of "a lot" of new methods, too rapid
change of curriculum and a shortage
of skilled teachers. The terms of refer-
ence of the Committee of Inquiry are:
"To consider the teaching of mathe-
matics in primary and secondary
schools in England and Wales with
particular regard to the mathematics
required in further and higher educa-

tion, employment and adult life gener-
ally and to make recommendations".

But workers in the field of numer-
acy and maths instruction greet these
developments unenthusiastically. Jo
Stephens of the DES Mathematics
Inspectorate points to some historical
antecedents of the present upsurge of
interest in mathematics.

In 1954 the Mathematical Associa-
tion reported: "The standard of
mathematical ability of entrants to the
Trade Courses is often very low".

In 1925, the Board of Education re-
ported: "It has been said that ac-
curacy in the manipulation of figures
does not reach the same standard which
was reached 20 years ago. Some em-
ployers express surprise and concern
at the inability of young persons to
perform simple operations involved in
business".

In 1912 an LCC report noted:
"There is a prevailing opinion that
London elementary school children of
today are slower and less accurate in
computation than they were ten years
ago". And in 1876 the Mathematics
Inspector reported: "In arithmetic I
regret to say worse results than ever
before have been obtained—this is
partly attributable no doubt to my
having framed my sums as to require
more intelligence than before . . .".

The problem, then, is not a new one.
Seen in this light the current focus on
numeracy is simply a political football
to be pursued by the media and
government as it suits them.

Blanket blame of teachers, attacks
on mixed ability classes and an abstract
concern with 'standards' characterise
the debate so far. Some members of
the Expenditure Committee are alive
to the fact that a person's numerical
skills bear little relationship to the re-
quirements of the available jobs.
"Unqualified school leavers competing
for the diminishing pool of jobs at
below craft level find that they lose out
to the qualified school leaver who is
eager to take a job below his (sic)
capability rather than be unemployed".

In other words, in a buyer's market,
employers are selecting the qualified
school leaver for jobs that would nor-
mally go to the unqualified leaver. The
unemployment problem is not due to a
lack of competence of the workforce.
Making people more numerate may
help them to compete more effectively
but it will not increase the number of
jobs. To imply that it will, however
indirectly, is to make a scapegoat out
of the schools.

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Nevertheless, Dame Kathleen's re-
port does indicate that there is room
for improvement in the mathematical
abilities of school leavers. Where *does*
the problem lie? The teachers them-
selves should surely know best.

Most numeracy teaching is based in
fifteen Colleges of Further and Higher
Education in the London area. The
problems presented to teachers are ex-
tremely varied—as are the students. A
major facet is that the problems of
numeracy and literacy are intertwined.
There are very few people who can
work with numbers without being able
to read and as such many numeracy
problems are handled within the much
larger literacy programmes. The ma-
jority of remaining students are young
adults who either have been referred to
numeracy courses from programmes
like Manpower Services Commission's
Training Opportunities Scheme or who
feel the need to improve their compu-
tation skills for various courses ranging

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*Good performance in maths needs close,
individual attention*

from craft apprenticeships to office work.

Linda Major an Advisory Teacher in numeracy based at Westminster College, says that students generally have little trouble with addition but do have certain problems with other operations. Frequently, she says, students will be able to use subtraction in the act of handling money but will have difficulty in working it out on paper. Major feels that some part of the traditional curriculum such as long division and fractions are of little practical use in adult life and makes no attempt to teach this.

Relevance is crucial to the approach. The students are riveted when it comes to the question of tipping but it is another matter when percentages are taught. (Literacy methods are similar. Jud Stone of City and East London College uses materials created by the students themselves. Instead of reading texts of little immediacy students learn to read their own words. The method places far more emphasis on writing than reading.) Major says that the goal of fluent single digit addition and multiplication along with a knowledge of how to compute with larger numbers using the ordinary place value algorithm is attained when there is adequate staffing.

Numeracy problems at this level, where for varied reasons, young adults have not learned to write, read and do arithmetic seem to be quite capable of solution. The primary obstacle is financial. More teachers, more space, workshops for teachers and students to exchange ideas and methods, more money. In Hackney with a population of 194,000 there are an estimated 15,000 people needing this kind of assistance. The present numbers of paid staff and volunteers help only 300 students. This is not a crisis but a well defined need on the part of 890 of the population of Hackney. Current expenditure is too low. The Adult Literacy Support Services promises some additional volunteer assistance

but it is doubtful that without proper training, proper space, proper materials, in short, proper organisation, that such an intervention can be effective. Some workers question the wisdom of basing a programme on unpaid labour but in the current situation they are not going to refuse help in any form.

At a higher level, more fundamental questions can be raised. University graduates in general and scientists in particular have all experienced difficulties when confronted with mathematics. According to Emilio Segrè, Enrico Fermi attended a conference at Bohr's institute on the weak interactions. At the end of two hours of symbols the lecturer concluded, "And that is Fermi's theory of the weak interaction". Said Fermi, "It was the only part of the lecture I understood".

Mathematics has had in its history a long dishonourable thread of mystification and inaccessibility going back to Plato. The most militant opposition to this tendency that I know occurred in Germany in the 1890s where engineering students organised demonstrations against the trend towards metamathematics then dominant in teaching at the university level.

Professor Morris Kline of the Courant Institute in New York has written a series of books and articles criticising the mathematics professionals for elitism and obscurantism in the teaching of mathematics, a position supported by Richard Courant himself when he was alive. The tendency to disconnect mathematics from its roots reaches down to the primary grades where number in the abstract begins to present difficulties to some students. The difference between tipping and percentage is a real one and problems with numeracy and problems with mathematics are connected.

Additional criticism of mathematics along these lines is starting to come out of the analyses of sex differences in educational achievement. Alison Kelly, a lecturer in sociology at Manchester

University says that as well as social pressures in all their forms that are exerted on girls to avoid science and maths there is the problem of the stereotypical image of science and maths itself—cold, remote and abstract.

Kelly and other analysts assert that women's rejection of mathematics is far more active than the usual portrayal of women as passive victims of a social role. "Women reject mathematics and the sciences as military and industrial activities bearing no relationship to helping activities or being able to explain oneself to oneself". If this is true of large numbers of women it is interesting to speculate on the possible effects of a major government effort to recruit more women to engineering, maths and science.

A different approach to the well-educated person's problems with mathematics was tried last year by Larry Buxton of the DES Maths Inspectorate. Recognising that there were important emotional responses to mathematics Buxton attempted to deal with these directly using the methods of psychotherapy. He worked with a group of six adults for 24 sessions and with three individuals for 12, 24 and 36 sessions respectively.

On the basis of the experience Buxton is convinced that the central problem is as much emotional as cognitive. "The exploration of emotion is not supposed to be related to in mathematics yet it is the subject with the most charged emotional content". Numeracy teachers deal routinely with problems on this level although it seems unlikely that widespread intervention based on individual counselling is feasible. But Buxton thinks that two widely accepted practices in mathematics instruction are open to question.

The first is the use of timed tests. The present curriculum confuses the need to work hard with the need to work fast, he says. Good performance in mathematics needs close, concentrated undivided attention and students must not feel rushed. The second practice involves complex attitudes towards authority. An interrogative approach in the classroom based on categorical right or wrong answers can be threatening and off putting to many students. This combination of time and authority was a recurring theme for the people with whom Buxton worked.

So there are many directions in which the teaching of mathematics can be improved. But a barnstorming attitude based on the false perception of a 'crisis' and on weak analysis of the problem is going to achieve very little. If the government wishes to improve the mathematical level of its school-children it could do a lot worse than to listen to those teachers most directly confronted with the problem. □

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correspondence

GMAG should look to NIH

SIR,—I write to enquire whatever happened to the public debate on changes in the regulations for genetic manipulation experiments? The notion of rationalising genetic manipulation containment by measuring the risk in each experiment was put forward in the columns of *Nature* last month. A public meeting was promised and was indeed convened in London on 21 December. This meeting could be called neither public nor a debate and achieved little or nothing. Views were expressed by members of GMAG, both on the panel and in the audience, that revealed strong political polarisation but little in the way of scientific evidence supporting either the present guidelines or possible alternatives. From the body of the meeting the only view which came across strongly and repeatedly was the indignation of many scientists at the classification of experiments involving the manipulation of *E. coli* DNA in *E. coli*. A host of important questions concerning the proposed new systems of evaluating the necessary containment for particular genetic manipulation experiments were not raised at this meeting. Even where one or two points were raised, time for discussion was inadequate. It is safe to conclude that there has been no proper forum for public debate.

By contrast to events in this country we have the example of the way in which the NIH guidelines have recently been revised in the US. Irrespective of whether one agrees or disagrees with the new NIH guidelines one must admire the broadly based public debate, the informed political comment and the rapidity of the process of modification of the proposals in response to debate and comment. Moreover the whole process, up to and including publication of the new guidelines, is freely available in the Federal Register. We could, to our advantage, learn from those events.

The need to change the genetic manipulation guidelines in Britain is just as pressing as was the need in the US. Our present guidelines are illogically restrictive. The levels of containment imposed upon genetic manipulation experiments in Britain have from the beginning exceeded those required in the US. This difference taken together with the lack of availability of physical containment facilities in this country has meant a severe handicap for British work with systems designated as requiring high containment. The introduction of the new NIH guidelines has widened the gap between containment required in the US and that required in Britain to such an extent that it is now impractical to continue a wide range of genetic manipulation experiments in this country. On a global basis it perhaps matters not whether Britain is involved in genetic manipulation research. For

Britain however, the consequences of missing out on a revolution in biological technology and in losing a generation of the best young scientists who wish to be trained in that area will be dire.

If the guidelines for genetic manipulation experiments in Britain are to be changed then the change must be effected rapidly. There is no sign of swift action on the Brenner initiative nor of an informed debate. Looking again at the global picture, should we be so insular as to think that we should repeat a process which has been painstakingly conducted in the US. Biological risks by their very nature do not respect national boundaries. We would be wise to look carefully at the guidelines accepted by the majority of scientists working with genetic manipulation and by the nation within which they carry out their work. I think that we could, without endangering either workers in the field or the public at large, adopt the philosophy of the NIH guidelines and indeed, with certain modifications to British conditions, the letter of those guidelines. The NIH guidelines remain largely empirical but they are based on extensive experience. In science we do not look at national origins in evaluating ideas or experimental results. I trust that in this case that neither national pride nor political ideology will prevent serious consideration of the NIH guidelines as a suitable model for genetic manipulation work in Britain.

Yours faithfully,

ALAN R. WILLIAMSON
*Institute of Biochemistry,
University of Glasgow, UK.*

The status of safety officers

SIR,—In your editorial "Smallpox: ignorance is never bliss" (11 January, page 75), you advocate a new breed of medically qualified safety officers, universally respected. Whilst I agree that a properly professional approach to laboratory safety is required, I suggest it would be inappropriate to require medical qualifications for safety officers.

Your suggestion that "a safety officer should always be a doctor" is ill considered. The example upon which you seem to have based the proposal is misleading. Dr Mark Darlow was in a unique position at MRE Porton. He was successful because he was the right person for that particular job.

There has been a predictable reaction in the press to the whole sad saga of Birmingham. We must use the impetus of public concern to improve our scientific safety position. It is undeniable that our scientific safety has left much to be desired and that many of its practitioners have been ineffectual. We should introduce a system for recognition of professional safety officers combined with a worthwhile career structure to encourage people in

whom both the general public and the scientific community can feel confident.

The status of safety officers should be assured by their employers—who are ultimately responsible for safety under the Health and Safety at Work Act (1974). Safety officers are charged with obligations to ensure the observance of statutory regulations and safety codes. They should work in conjunction with a safety committee constituted to represent all groups of employees from academic staff to ancillary staff.

Thus it will only be when properly qualified and professionally accountable safety officers are employed and given the opportunity and incentive to carry out research and to get on with the job of safety in science, that we could feel that we have learned the lessons so apparent in this latest tragedy.

Yours faithfully,

M. R. BAILEY
*National Institute for Medical Research,
London, UK.*

Reason not ruction

SIR,—In your issue of 21/28 December, page 753, Dr Kenneth Mellanby quotes a secular hymn from a Socialist Sunday School in Glasgow in the 1920s:

"By nature not nurture we'll rise to the skies

The means of production we'll nationalise."

This is curiously like the refrain from a revue song by A. P. Herbert, which I quote from memory:

"By reason not ruction we'll soar to the skies

The means of production we'll nationalise

And rapture surprising we'll bring within range

By nationalising the means of exchange."

I prefer APH's version.

Yours faithfully,

O. MAYO



A. P. Herbert: British humourist

news and views

Periodical cicadas

from Robert M. May

IN 1970, Princeton University conferred an honorary degree on Bob Dylan. The graduation ceremony coincided with the local emergence of periodical cicadas, which appear in vast numbers every 17 years, and the air was dense with their bodies and their song. Dylan recorded the occasion ('Day of the Locust' on *New Morning*, Columbia PC-30290):

These cicadas (not locusts; Dylan's honorary degree was in Letters not Science) are a unique biological phenomenon. They comprise three species in the genus *Magicicada*, and have the longest life cycle known for any insect. Found throughout the eastern United States, the nymphs live 6 to 24 inches below the ground, where they suck juices from the roots of forest trees. Mature nymphs emerge, assume their familiar adult form, mate, lay eggs and die within the same few weeks of every seventeenth (in the north) or every thirteenth (in the south) year. The synchrony is astonishing: although different 'broods' in different regions may be out of synchrony by several years, in any one population essentially all the animals are the same age, and the three different species are invariably synchronised whenever they co-exist (as they commonly do). As far as anyone has been able to tell by conventional taxonomy or by the song of the males, each of the three species exists with both a 17-year and 13-year life cycle.

The difficult questions that are posed by the existence of these remarkable creatures are lucidly set out in the classic papers of Lloyd and Dybas (*Evolution* 20, 133; 466; 1966). There are two basic problems. First, how did such a cycle, with so long a period, originally evolve? Second, how is the clear-cut periodicity and the synchrony between co-occurring species maintained?

The second question is more easily answered than the first. Given that there already is a long life cycle and a

nearly-perfect periodicity of emergence, the cicadas can effectively escape predation. Above-ground predators such as birds, who have no analogous resource in the long intervals, are satiated, and below-ground predators, such as moles, are unexpectedly deprived of food by every periodical emergence. The resulting population densities achieved by periodical cicadas are one or two orders of magnitude greater than those of their more stodgy relatives that appear each year. Lloyd and Dybas estimate that the nymphs may typically attain densities of one ton per acre. The selective pressures are for doing well at high population densities, even at the cost of being conspicuous and sluggish ('predator-foolhardy' is Lloyd and Dybas' term). Any mutant, migrant or developmentally different individual who fails to emerge with the majority will almost certainly be snapped up by predators. Lloyd and Dybas review observations and experiments bearing this out: typically the cicadas that emerge during the first few days are all eaten by birds; in 1889 Marlatt transported large quantities of eggs to an area where the adults that emerged in 1906 were out of phase, and the entire colony of more than 10,000 individuals was effortlessly consumed by grackles. In brief, the long-period cycle enables the cicadas to saturate their predators, which reciprocally helps to stabilise the cycle as individuals appearing earlier or later are gobbled up.

This conspicuous association between predation and periodical behaviour has led many people to speculate that the magic numbers 13 and 17 arise because they are prime, thus preventing any possible subharmonic resonances by the predators. Robert MacArthur remarked that it may be the only application of number theory, as such, in mathematical biology. I am surprised nobody has yet suggested that the prime number 11 is missing because it permits resonances with the sunspot cycle!

Two recent papers have developed some of these notions more formally,

providing useful insights.

Hoppensteadt and Keller (*Science* 194, 335; 1976) present a mathematical study whose essentials are simple. The cicadas have a life cycle of length L years. All 'broods' may be present, that is, the periodical emergence is not assumed. Each year the nymphs in the appropriate brood emerge and become adult, with each surviving adult producing an average of f offspring. Nymphs belonging to the other broods suffer some mortality, with a proportion α surviving into the next year. The newly hatched nymphs become established underground, subject to the constraint that the total number of nymphs present in the ground in any one year is limited to be not more than some carrying capacity K . Finally, the adults suffer predation, up to a saturation level that depends on the predator density: if the number of cicadas is below this saturation level, all are eaten; if above, the excess survive to reproduce. The predator density depends on the previous year's density (reduced by a mortality factor R) and on the previous year's recruitment (proportional to the number of cicadas consumed that year). This dynamic model of the interaction between cicadas and their predators can have three distinct outcomes. If predation is too severe, the cicadas cannot survive, and the system collapses. If predation is relatively unimportant, a 'balanced' equilibrium in which a constant number of cicadas emerge each year is possible, and seems to be the only stable state. Most interesting is the case where predation is of an intermediate strength, such that a balanced state is not possible, but a periodical state (with just one brood, emerging every L years) is possible. The criterion for this intermediate situation to hold is that

$$\left(\frac{AF}{1-F}\right) > 1-R > \left(\frac{AF}{F-1}\right) \frac{R^{L-1}(1-R)}{(1-R^L)}$$

Here R and L are as defined above, $F=fa^L$ is the average number of offspring produced, and A is the efficiency

at which consumed cicadas are converted into predator biomass. The mechanism underlying this intermediate case is not simply that the high cicada density associated with the periodical state saturates the predators while the balanced emergences cannot (remember that in this model higher cicada densities will generate higher predator densities), but rather that the longer periods between recruitment in the periodical state make it harder for the predators to keep their density high (hence the factor R^L on the right hand side of the equation). The above criterion is a rather delicate one to satisfy, and large values of L help to do so. Hoppensteadt and Keller arbitrarily choose parameter values which produce beguiling figures in which synchronised, periodical behaviour is manifested once the life cycle L exceeds 10 years, with balanced annual emergences for L less than this.

Hoppensteadt and Keller's model is valuable in showing how periodical behaviour can emerge as a stable phenomenon, provided that predation is of some intermediate intensity. The mechanism involves an explicit interplay between resource limitation (without which the system would either collapse or run away) and predation (without which the system would settle to a balanced state). The conclusions are not sensitive to Hoppensteadt and Keller's assumption about the dynamics of the predator population. If I replace their dynamically varying expression by a fixed predator population of magnitude P , the same three cases arise, with the interesting intermediate situation pertaining if

$$\left(\frac{1-\alpha^L}{1-\alpha}\right)\left(\frac{F}{F-1}\right) > \frac{Ka^L}{P} > \left(\frac{F}{F-1}\right)$$

This expression has the same general character as Hoppensteadt and Keller's. In reality, the predator population is neither entirely independent of the cicadas, nor entirely dependent on them. It is therefore reassuring that the essentials of this mechanism for producing synchronised, periodical emergences are independent of the details.

Bulmer's (*Am. Nat.* **111**, 1099; 1977) study pays more attention to competition among the nymphs under the ground. Motivated by the suggestion that periodical emergences can be produced by competition that is more severe between than within year-classes, Bulmer considers a mathematical model without predators. He finds balanced solutions if competition is fairly uniform, and periodical solutions if inter-brood competition significantly exceeds intra-brood. When the effects

of predation are added (assuming a constant predator population), a conclusion similar to that of Hoppensteadt and Keller is reached: low levels of predation leave the balanced solution stable, but intermediate levels destabilise the balanced solution and produce a periodical one. In his discussion, Bulmer flouts the conventional wisdom, concluding that "competition between year-classes is the main factor likely to lead to periodical behaviour". This conclusion, however, depends crucially on whether inter-brood competition is indeed a lot more severe than intra-brood (as Bulmer assumes) or not (as Hoppensteadt and Keller assume). The facts surveyed by Lloyd and Dybas seem to me to speak for the later view: the fighting behaviour displayed by nymphs in defence of their cells suggests competition is significant; but there is great variability in size among nymphs of the same age ("the range of variation within a year is far greater than the mean difference between years", Lloyd and Dybas, *op. cit.*, page 473) which makes it unlikely that inter and intra-brood competitive interaction are greatly different.

Bulmer also considers the situation when two or three species of periodical cicada occur together, and shows that satiation of predators is a most effective mechanism for synchronising the emergences. He also compares his mathematical studies with information about the natural history of some other periodical insects, namely May beetles (which over most of their range are periodical with a 3-year life cycle, lengthening to 4 or 5 years in some regions of northern Europe) and the northern oak eggar (which has periodical behaviour with a 2-year cycle). Although these insects have much shorter cycle times, and less dramatic population behaviour, than the *Magicicada* species, Bulmer's idea of using them to help shed light on the evolution and maintenance of periodical behaviour is a nice one.

The studies by Hoppensteadt and Keller and by Bulmer both assume that the periodical cicada populations are limited by the resources available to the nymphs. Lloyd and Dybas contest this point. They suggest that more nymphs could usually be sustained in their environment, and that the overall population is limited by a fungus disease, *Massospora cicadina*, which afflicts adult cicadas; the resting spores of this fungus survive for decades, and thus amount to a form of 'predator' for whom 17 years is a short time. If Lloyd and Dybas' speculation is eventually borne out, it will constitute a striking example of the magnitude of a natural population being ultimately controlled by a disease (Anderson & May *J. Anim. Ecol.* **47**, 219; 1978).

There remains the fundamental question of how the 13 or 17-year cycle first evolved. Lloyd and Dybas survey two classes of explanation.

One envisions a periodical pattern first appearing in order to facilitate predator saturation, and then gradually lengthening. The problem here is that the very same mechanisms that maintain the periodical behaviour would select against any deviant cicada that tried to add a year to its cycle time; a cycle in which predator satiation played a crucial role could conceivably evolve to be shorter, but not longer. On this basis, the periodical cicadas seem to be sitting on an 'adaptive peak' to which there is no accessible approach.

The other type of explanation assumes an initially 'balanced' cicada population, controlled by predators and parasitoids, all of whose cycle times lengthened, perhaps for developmental reasons or perhaps driven by an evolutionary race between prey and predator. Once the cycle time L attained in this way becomes long enough (in the sense specified by the equation above), the balanced configuration can abruptly become unstable and yield a periodical one. Moreover this discontinuous change in the dynamics of the system could spell extinction for some of the previously key parasitoids, thus making the situation observed today all the more enigmatic. This is pretty vague stuff, but there is at least some evidence that cycle times for insect prey and predators can be long. Other species of forest cicada for which the life cycles are known have 7-year cycles; long cycle times may well be common among cicadas with more or less 'balanced' behaviour. Among their predators, the large Australian cicada-hunting wasp, *Exeirus lateritius*, has facultative dormancy of 2 or 3 years, and some of the parasitoids of the wheat blossom midge, *Sitodiplosis mosellana* (which itself has a dormancy underground of up to 13 years), exhibit dormancy periods of up to 8 years. Recent work on models for host-parasitoid systems with mixed developmental periods for the hosts and with parasitoids whose cycles are submultiples of their hosts' (MacDonald *Math. Biosci.* **31**, 255; 1976; Beddington, Free & Lawton *Nature* **273**, 513; 1978) lay the foundations for a more quantitative exploration of these ideas.

Finally, a kind of experimental test between the above two general explanations is possible. On the first class of explanation, we would expect the 13-year cicadas to have appeared first, and the 17-year ones to have evolved from them. On the second class of explanation (which acknowledges that once periodical behaviour becomes established, cycle times can only evolve to be shorter), the 13-year cicadas would

be derived from the 17-year ones. White and Lloyd (*Am. Midl. Nat.* **94**, 127; 1975) have shown that the 13-year cicadas have five instars (the last of 4 years' duration), while the 17-year ones have an extra, sixth instar of 4 years' duration. If the second explanation is correct, the 13-year cicadas evolved by dropping an instar, and they should be replacing the 17 year ones whenever they are in contact. Such regions of contiguity exist, for example, in the Ozark mountains, and detailed studies of future emergences in this region will be interesting. This touches on a practical problem in this research.

namely that, whatever their significance in the world of predators and parasitoids, 13 and 17 years are much longer than the time scale for most research grants and tenure decisions.

To summarise, it is hard to see how periodical behaviour can evolve. In particular, the Hoppensteadt-Keller criterion can only be satisfied by a delicate juggling of the pertinent parameters. But it should be remembered that the periodical cicadas comprise only 3 of some 1,500 cicada species; an easy explanation for the phenomenon would be an embarrassment!

□

Testing quantum chromodynamics

from Frank Close

OF the four natural forces only electromagnetism and gravity are very well understood. Theoretical developments during the past few years have produced candidate theories of the other two natural forces—the weak force of radioactivity and the strong nuclear force. 1978 saw the dramatic and extremely beautiful experiment at Stanford, California which discovered parity violation in electron-proton scattering (*News & Views* **274**, 11, 1978). This provided significant evidence supporting the model of the weak interaction developed over seventeen years by Glashow, Salam and Ward and by Weinberg and which became a candidate theory following work of 't Hooft in 1971. For the first time we appear to have a theory of the weak force which violates no sacred principles such as relativity, unitarity and causality, is renormalisable (does not have unwelcome infinite probabilities in it), is being increasingly confirmed by data—and could even be correct.

In addition to having apparently uncovered another of Nature's secrets we have obtained a bonus, namely that the weak and electromagnetic interactions are not two independent phenomena but are intimately unified (an intellectual achievement comparable with Maxwell's unification of electricity and magnetism). The above success has been brewing for the past 5 years and has been well described in the scientific press (*Nature* **264**, 505; 1976; **273**, 99; **274**, 11; **275**, 267; 1978). Meanwhile, encouraged by this achievement much enthusiasm and effort has been devoted to developing

a theory of the strong force—the one remaining force without a satisfactory candidate theory.

An elegant theory of the strong interaction has been recently developed and is known as 'quantum chromodynamics'. It has all the desirable necessary theoretical properties outlined above for the weak theory, and indirect clues gathered over the past few years have provided qualitative support for it. What has been lacking is a quantitative test whereby the theory can be eliminated if it fails. Two experimental collaborations at CERN, Geneva, have performed such a test and the results appear to be in agreement with the theory. Hence 1978 may go down in history as the year in which two of Nature's forces were at last revealed.

A theory of quark interactions

Quantum chromodynamics (QCD), the theory of the strong interaction, is almost identical to quantum electrodynamics (QED), the theory of the electromagnetic interaction. There is one minor difference between them which turn out to have far reaching consequences.

In QED the fundamental particles (leptons and quarks) carry electrical charge and couple to photons, the quanta of the electromagnetic field. The strength of the coupling is proportional to the charge of the particle. There is only one type of charge; like real numbers it can be positive or negative. Mathematically this is a U(1) field theory. Particles which do not contain electrical charge do not couple to the photon. Hence electrically neutral neutrinos do not couple to photons, nor do photons

couple to other photons directly as they are also electrically neutral.

It has been known for several years that quarks carry another type of charge called colour. Leptons (such as the electron) do not possess this charge. One immediately suspects that the strong force is somehow connected with this colour charge seeing that quarks do feel the strong force and also carry colour charge whereas the colour neutral leptons do not experience the strong interaction. One therefore creates a quantised field theory of chromodynamics—the interaction of coloured charge with 'gluons', the quanta of the chromodynamic field—and hopes that this will have relevance to the strong interaction. This is in complete analogy to electromagnetism. The gluons are the analogues of the photon and are massless, vector (spin⁺) particles.

The one essential difference between QED and QCD is that whereas there is but one type of electrical charge in QED, the colour charge has three independent varieties (often called red, yellow and blue charges by analogy with the primary colours). Mathematically this three-fold charge yields an SU(3) field theory as against U(1) for QED. This has the consequence that gluons themselves carry colour charge whereas in QED photons do not carry (electrical) charge; for example a red quark annihilating with a yellow antiquark will yield an orange (red-yellow) gluon. There is a total of eight coloured gluons as against just one photon in QED.

Coloured gluons couple to any particle which carries colour charge. The gluons themselves have colour. Hence gluons can couple directly to other gluons. This essential difference from QED, where photons being electrically neutral do not self couple, yields profound consequences but for which our Universe would have been very different, or even nonexistent.

Asymptotic freedom

QCD makes specific predictions as to the strength of the quark to gluon coupling. Its absolute magnitude is not understood (just as no-one has yet understood why the electromagnetic coupling has the magnitude $\alpha=1/137$). However, the coupling is predicted to have an effective dependence upon the interquark distance. This dependence has been confirmed in recent experiments. To illustrate what we mean, first recall the more familiar case of electromagnetism.

When a charge is placed in a dielectric it polarises the surrounding material. The effective strength of this charge, as measured by the force on

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a second test charge, will depend upon the separation of the two charges because when the separation is less than the intermolecular spacing the full effect of the charges will be felt without any dilution from the polarisation of the surrounding dielectric whereas at larger separations the polarisation dilutes the charge density. If we insist that the force is Coulombic, then we will be forced into concluding that the effective charge has increased at short distances.

The same phenomenon obtains in a vacuum where an electron surrounds itself with a cloud of virtual electron-positron pairs. A detailed study of QED confirms this heuristic picture: the value of α is not a constant but increases at short distances.

In QCD the prediction is precisely the opposite: the effective coupling decreases at short distances. This is essentially because the gluons now carry charge themselves (in contrast to the photons of QED) and tend to transport the charge of the source away from the source and into the surrounding cloud of virtual quark-antiquarks and gluons.

Hence if a very high frequency short wavelength beam probes the space-time structure of the proton at short distances, it will see a collection of effectively free quarks. This phenomenon was discovered ten years ago and was somewhat paradoxical since if quarks were indeed free then why did they not float freely out from the proton?

QCD appears to provide the answer to this paradox. It predicts that the quarks are free at asymptotically short distances, as observed. As the quarks are pulled apart so the force increases. Hence at large distances the inter-quark force is large and they are confined in the proton. This property of the quarks has suggested the comparison of the proton to an ideal prison—perfect freedom inside but no-one can escape.

Testing QCD

The above is a pleasing resolution of a paradox—free confined quarks—but is hardly sufficient to confirm a theory. What are needed are quantitative predictions to test. Last summer a collaboration from Aachen, Bonn, CERN, London, Oxford and Saclay produced data on very high energy neutrino-nucleon interactions at the CERN-SPS which appear to provide the first dramatic quantitative test of the theory (Bosetti *et al.* *Nucl. Phys.* **B142**, 1; 1978). A recent paper from a CERN-Dortmund-Heidelberg-Saclay collaboration (de Groot *et al.* CERN report) has provided indepen-

dent confirmation of these results.

What has been shown is that there are gluons as well as quarks in the proton. The gluons have been shown indeed to be vector particles (like the photon and as required by QCD). The short distance interquark force cannot be probed to infinitesimal distances because the experiment is limited to finite energy. By tuning the energy one can measure the distance dependence of the quark-gluon coupling. The quantitative behaviour was found to be as predicted by the theory.

These are all solid clear cut tests of the theory. There are also some hints in the data that may provide support for the prediction that the gluons can couple to each other (as a result of their carrying colour charge) but this will require much more study before being fully proved.

Another test of the theory involves electron-positron annihilation. At high energies the particles produced appear to come in two jets, as expected if they are the products of a fast quark and antiquark that have been produced in the e^-e^+ annihilation. If the e^-e^+ produce a massive vector meson like the ψ/J at 3.1 GeV or the Y at 9.4 GeV, then QCD predicts three jets. This is because the meson turns into three gluons, three being the minimum number allowed by the various conservation laws. The ψ/J is too light to allow a good test of this prediction. Studies of the more massive Y at DORIS, Hamburg, are beginning to provide data supporting the three jet prediction. These tests will be high on the list of priorities of the early physics at PETRA, the new high energy electron-positron machine at Hamburg.

In 1979 we can hope to see QCD established as the theory of the quark force. If, as seems probable, it is confirmed then we will have theories of weak, electromagnetic and strong forces. The former pair are already known to be intimately linked and unified. Their similarity in structure to QCD, if verified, hints at more than coincidence and promises that all three may be related or unified.

Ideas on this are already being developed in anticipation. Experimental confirmation of QCD will be a milestone as another of Nature's forces will have been revealed. It seems possible that the dream of synthesising all of the natural forces may soon be realised with potentially profound consequences.

There is great optimism that the experimental discoveries of 1979 will be pointing us down this road. This would be an apt celebration of Einstein's centenary as it was his genius that gave us the theory of the

remaining force, gravity. Furthermore he spent a substantial part of his later life attempting to build a unified theory of the natural forces. If we are indeed on the verge of verifying QCD, and if unification with the electro-weak interaction is next, then the final step will be to include gravitational interaction. $\square$

Immunology of malaria

from F. E. G. Cox

THE possibility of producing a vaccine against malaria, probably the most important disease in the world, is one of the major challenges facing biomedical scientists today. This is a field for optimists for, as the pessimists point out, there is no immediate possibility of such a vaccine. On the other hand, over the past few years progress in our understanding of the immunology of malaria has been phenomenal and it is important at this point to take stock and to assess the possibilities of vaccination and also, assuming that an effective vaccine can be produced, the problems inherent in making it acceptable. The World Health Organisation has encouraged most of this recent work and the purpose of a recent workshop* was to review our present state of knowledge of this subject.

In malaria, sporozoites injected by a mosquito circulate in the blood for about 30 min before entering the liver to undergo the first stage of multiplication. Invasive stages, the merozoites, flood from the liver and invade red blood cells in which further multiplication occurs, resulting in more merozoites, and eventually sexual stages, gametocytes, form and these are taken up by the mosquito in which the life cycle is completed. Immunological responses to all stages of the life cycle have been demonstrated but a major problem is that these responses are stage-specific. At present, there are three main candidates for the preparation of conventional vaccines; sporozoites, merozoites and gametocytes. The sporozoite is an obvious target and the possibility of an anti-sporozoite vaccine, pioneered by R. S. Nussenzweig (New York), has been adopted by the US Navy. Successes have been reported using sporozoites inactivated

*A Workshop on the Immunology of Malaria was held at the National Naval Medical Center, Bethesda, Maryland on 2-5 October, 1978. It was sponsored by the Naval Medical Research Institute, USAID and the World Health Organisation.

in various ways in several host-parasite models. The main advantage of the sporozoite vaccine is that it does not need an adjuvant while the disadvantage is that it requires a number of immunising injections.

Merozoite vaccines (developed by S. Cohen, London) are also effective and the material necessary for preparing the vaccine is now easily and safely obtained but adjuvants are required. Gametocyte vaccines (R. W. Gwadz, National Institutes of Health, Bethesda) prevent fertilisation within the mosquito but depend on the vertebrate host experiencing an infection. This kind of vaccine is effective and academically interesting but hardly acceptable in its present form. In addition to these conventional specific vaccines there is also evidence that nonspecific factors play a part in immunity against malaria and may involve the natural killer (NK) cells now being implicated in tumour immunology (A. C. Allison, Clinical Research Centre, Harrow; H. Wigzell, University of Uppsala).

It is obvious that the vaccines in use at present need to be improved and considerable effort has gone into exploiting the possession of stage-specific antigenic differences and it is in this area that biochemists are coming into their own. The antigens of *Plasmodium knowlesi* from rhesus monkeys have been well studied and merozoite antigens have been analysed by J. A. Deans (London), sporozoite antigens by E. Nardin (New York) and blood stage antigens by R. Schmidt-Ullrich (Boston). However, this work has proceeded little further than the preparative stage at present and work with *P. falciparum* is no farther advanced (R. J. M. Wilson, London).

The final part of the meeting was devoted to the practicability of developing a vaccine suitable for use in humans. The need for carefully controlled trials and the difficulties inherent in these were stressed by I. A. McGregor (Gambia) and the possibility of having to accept some risks and only partial success was raised by R. D. Powell (University of Chicago, Chicago). Because of the need for safety and the difficulties in finding volunteers and naive populations in endemic areas, the successful development and perfection of a vaccine may present even greater problems than discovering one.

It may be that asking a group of malaria immunologists if they believe in a vaccine is like asking the College of Cardinals if they believe in God but the general consensus of opinion from the meeting was that scientists should be asking when a malaria vaccine could be developed, not if, and this opinion must justify the World Health

Organization's faith in such a possibility. □

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The surface photoelectric effect

from John Pendry

ONE of the theoretician's favourite models of a solid is a box in which the potential is constant so that the electrons move around freely. The mathematical simplicity gained by neglecting atomic scattering enables a comprehensive treatment of the problem to be made; in particular the surface of the box can be studied in detail. There is a competition between the electrons' desire to stay inside the attractive potential of the box, and the tendency to spill over the surface and so reduce the kinetic energy. The situation is made doubly complicated because of the charge on the electron which means that as the electron wavefunctions are modified, the potential changes as well. A self-consistent determination of the electronic wavefunctions and the surface potential must be made to discover the height of the potential barrier at the surface, and its shape, particularly its width, which reflects the degree to which the electrons spill over the surface. This surface potential is important for certain applications such as the study of chemisorption at a surface, or electrical properties of surfaces. An alternative way of viewing the electrons' response to the surface is to treat them collectively as a dielectric medium which screens the bare surface potential of the original box without the electrons. This collective view is particularly appropriate when a time-dependent perturbation, such as a photon, disturbs the system. The electrons are caused to oscillate collectively and are known to exhibit characteristic resonances at the surface-plasma frequency. This frequency is determined by the details of the surface, as is the distribution of the charge that takes part in the collective oscillation. Again these surface plasma oscillations dictate some of the basic properties of the surface.

Surprisingly enough, it is believed that this idealised model of free electrons is quite close to the truth in simple metals such as sodium, potassium, magnesium or aluminium. It

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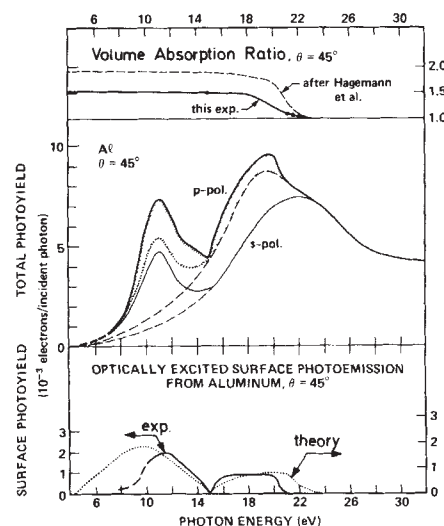


Fig. 1 The results of Petersen for s and p polarised photoemission from an aluminium surface.

fails of course when the atomic character of the electron orbitals persists in the solid, as in the transition metals. The existence of a large body of theory for this tractable model, and the physical realisation of the model in several easily available materials could be expected to lead to a rapid growth in our understanding of the surface potential and the surface plasma oscillations. This has not been so. Interpretation of the major experimental technique, photoemission, has many complications and it has not been easy to vary enough parameters to get a comprehensive picture. Recently Petersen and coworkers (*Zeitschrift für Physik* **B31**, 171; 1978 and *Phys. Rev. Lett.* **41**, 1314; 1978) have used a synchrotron to provide a source of polarised photons whose energy can be varied at will in the 0-25 eV range. It seems that experiments with this source can confirm many of the theoretical predictions made in recent years.

In an extremely simplified picture of photoemission, the emitted current I of electrons is proportional to

$$I \propto \sum_f |\langle \psi_i | \mathbf{A} \cdot \nabla V | \psi_f \rangle|^2$$

where ψ_i is the electron wavefunction before excitation, ψ_f the wavefunction after excitation, $\mathbf{A}$ is the A-vector of the electromagnetic field, and V is the step in potential at the wall of the box, which confines the conduction electrons inside the box. It is commonly called the surface barrier, and in the free electron model at least is a function only of the coordinate normal to the surface. We can easily understand the occurrence of both $\mathbf{A}$ and ∇V in the expression for the current: the photon described by $\mathbf{A}$ provides the energy to excite the electron, but

carries hardly any momentum, ∇V on the other hand is a source of momentum normal to the surface and allows both energy and momentum to be properly conserved in the transition. I should add that in a rigorous treatment other terms appear that are due to inhomogeneity of $A(r)$ at the surface. Obviously our equation provides a means of checking both the variation of charge density at the surface (which determines V) and the dielectric properties (which determine A , given the conditions of incidence).

Complications arise on two accounts: (1) The potential inside the solid is not perfectly constant and ∇V will have volume in addition to surface contributions; (2) the critical parameter in the equation above is the angle between A and the surface normal. Any roughness of the surface will spoil the simple predictions. Complication (2) is much more critical than might have been suspected: even small inhomogeneities in the surface can excite the surface plasma oscillations which propagate away from the inhomogeneity and change the A vector over a large area.

Both (1) and (2) have to be subtracted before comparison with theory can be made.

By changing the polarisation of the light from s (A_s parallel to the surface) to p (A_p perpendicular to A_s) the true surface effect can be switched on. Figure 1 shows Petersen's results for s and p polarised light. When the s results have been renormalised to correct for the larger reflectivity of s light they give the contribution only for effects (1) and (2) to the p polarised results. The remainder results from the $A \cdot \nabla V$ terms which is of course zero for s light. The lower panel in Figure 1 shows comparison of the corrected experiments with theory (Endriz *Phys. Rev. B7*, 3464; 1973) taking account of surface plasmon effects in addition to the simple term shown in the equation. The results are encouraging but reveal that theory has some way to go before a complete description is available. At least the new generation of experiments are now providing the checks on theory required to stimulate further advances.

□

Non-ideal plate tectonics

from Ron Oxburgh

THE general character of the large scale tectonic plates that make up the surface of the Earth is by now fairly well known. So too are their present day motions as they separate, allowing warm ductile rock from below to well up and congeal onto their trailing edges; as they slide past each other, or as they converge, on sliding beneath another to be assimilated once more into the Earth's mantle. Three lines of evidence may be used to constrain these motions: spreading rates, earthquake slip vectors, and the trends of transform faults; all are employed in a recent analysis of plate motions by Minster and Jordan (*J. geophys. Res.* **83**, 5331; 1978).

They estimate spreading rates from the distance of a characteristic magnetic signature on the ocean floor (anomalies 2 and 2') from the ridge crest at which it was formed; the rates are thus averaged over a narrow and well constrained time interval of about 3 million years. Earthquake slip vectors and transform fault trends allow the direction of relative motion between two plates to be determined; the former are established by studies of the first motions associated with a particular earthquake observed in

different parts of the Earth, and constrain or define the relative motion on the plate boundary where the earthquake occurs. However, the best directional constraint is provided by transform faults; they must lie along small circles parallel to the direction of movement between the two plates, as along them plates neither separate nor converge.

Minster and Jordan use 110 estimates of sea-floor spreading velocity, 78 transform fault azimuths and 142 earthquake slip vectors to construct a world-wide, internally consistent model for motions between 11 surface plates. They show that this new data set provides a model which is free of most of the inconsistencies of earlier syntheses and with one major exception, fits all observations extremely well. The exception is the boundary of the Indian plate with the Antarctic and Pacific plates where there are systematic misfits. Although this could be the result of observational errors the authors point out that it could also be the result of internal shortening within the Indian plate in a northwest-southeast direction at a rate of about 1 cm yr^{-1} .

Clearly one must hesitate before accepting an interpretation which involves an assumption diametrically opposed to that upon which the model was constructed in the first place, that

is, that plates are internally rigid. One must ask whether in that case the Indian plate is the only one undergoing internal deformation and, if not, whether the fit achieved elsewhere may not be fortuitously good.

The answer to these questions is probably that the authors are right. A somewhat improbable pattern of observational errors would be required; and, on the other hand, the Indian plate is sufficiently unusual in several other respects that it could be different in this way too; not only did it move northwards during the Mesozoic at, by plate tectonic standards, the breakneck speed of 17 cm yr^{-1} , but after the collision of the Indian and Eurasian continents continued to do so at a significant rate (the Minster and Jordan data suggest about 5 cm yr^{-1}). The interaction of the two continental masses must interfere with steady subduction along the northern boundary of the plate and give rise to anomalous stress conditions within it. It is worth pointing out that the plate is also the site of a massive and unexplained geoid anomaly—the geoid (the gravitational equipotential surface) is depressed about 110 m below its expected value.

The interaction of the Indian and Eurasian plates finds its most spectacular expression in the Himalayan Mountain chain. Very different in character, but apparently developed almost simultaneously, is the Tibetan plateau immediately to the north. The plateau is a lozenge-shaped flat area of over a million km^2 bounded by mountainous zones of folded and faulted rocks. The plateau was beneath sea level at the end of the Cretaceous period and has subsequently been elevated without significant folding to a height of about 5,000 m. There has been extensive young volcanic activity within it during the uplift and continuing to the present.

Relatively little is known, at any rate in the West, of the geology of this fascinating area. It is therefore hardly surprising that two separate groups, Molnar and Tapponnier (*J. geophys. Res.* **83**, 5361; 1978) and Ni and York (*J. geophys. Res.* **83**, 5377; 1978) have taken the opportunity of studying it by means of the new ERDA LANDSAT photographs and interpreting these along with slip vector information derived from earthquakes occurring within the area. Both arrive at the interesting conclusion that although in the Himalayas along the southern margin of the plateau there is thrust faulting, within the plateau there are north-south trending normal faults with the formation of rifts, and indicating an extension in an east-west direction. There is also strike-slip faulting in a WNW-ESE trend. It seems

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therefore as if the Tibetan plateau is responding to compression from the south not by folding but by a kind of lateral extrusion. This requires a ductile behaviour in the underlying crust and upper mantle, a condition which is compatible with an observed strong attenuation of elastic waves beneath the region.

Molnar and Tapponier take the further step to suggest that the ductile lower crust and upper mantle beneath the Tibetan plateau may be regarded as the working fluid in a massive geobarometer, the elevation of the plateau reflecting the level of stress transmitted to the fluid contained by surrounding but uncoupled blocks. The authors essentially propose the decoupling of a thin and more or less passive brittle, upper crustal layer from a ductile zone beneath, which experienced relatively homogenous crustal shortening. It does seem, however, that the amount of

isostatically compensated crustal shortening required to 'pump up' the Tibetan plateau to 5,000 m is very large (of the order of 100%) and this would involve a much larger strain in the overlying plateau than is observed.

Molnar and Tapponier may not have solved the problem of plateau uplifts—there are other comparable but smaller features such as the Colorado plateau—but they have refocused attention on the long standing problem of how stresses are transmitted through the lithosphere to produce localised strains. The deformation is clearly inhomogeneous and there is much to be learned about the formation and decoupling and interaction of small scale crustal and lithospheric blocks. The combined application of the methods of earthquake fault-plane solutions, surface geology, gravity, and elastic wave attenuation studies offer a promising way forward. □

lege). It turns out that food is often 'handled' rather than 'screened'. A long filamentous diatom colony, for instance, is manoeuvred by a copepod to be end-on to the mouth, then fed into the jaws and broken up like brittle spaghetti. Larger fragments vanish down the animal's mouth but smaller fragments drift away. In a feeding rotifer, a stream of water flows against the ciliated wheel, where different algal cells are accepted or rejected as they thump against the cone that leads to the rotifer mouth.

Data on what particles are removed from suspended mixtures also show that herbivores in the plankton do specialise to some extent but that the discrimination is fairly crude, usually by size or against the obviously scarcely edible. Copepods and rotifers discriminate more than do cladocerans.

The habit of swimming up by night and down by day (vertical migration) has various possible advantages. It hides zooplankton from predators that hunt by sight. But the habit can also result in a systematic food search and it also ought to let plankters lower the energy cost of respiration by putting them in the cold bottom water when not actively feeding. The conference was given strong evidence that all three mechanisms were, indeed, driving the habit, with a suggestion that predation was the most prevalent.

Water mites, which are apparently without serious predators, may migrate up and down, apparently in quest of food or optimum temperature. But plankton in tropical Lake Gatun abandoned a former pattern of vertical migration when their fish predators were removed. In Lake Llano in the Philippines *Chaoborus* is restricted to water deep enough to let it hide from fish at the dark bottom by day. In the 30 m high experimental chamber at Dalhousie University marine copepods went up and down when the water was essentially without food but abandoned their migrations to remain at an introduced layer of algae. In the Arctic Alaskan Toolik Lake, where there is no night in summer, there was no regular vertical migration but the animals adopted a depth which depended on temperature or their age and hunger.

Another way in which the larger zooplankton avoid predators appears to be by 'cyclomorphosis'. Specimens of some large Cladocera have a significant part of their total volume taken up by essentially transparent protuberances, a change of shape of the growing animal that can be expected to minimise the target presented to a fish

Life at low Reynold's number

from Paul Colinvaux

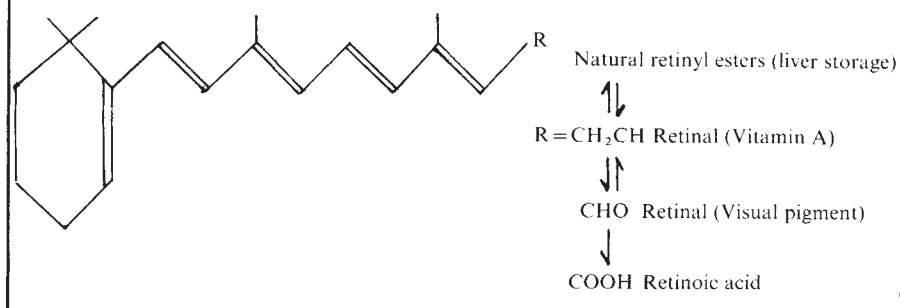
SMALL planktonic animals must feed in water which has properties a little like those we know in treacle, as the size and slow motions of the zooplankters make them live at low Reynold's number, at which liquid flow becomes laminar. One of the properties of laminar flow is that raking or stirring movements do not jerk particles through the water so that there is no easy displacement of suspended matter. Planktonic herbivores respond to this reality by generating a flow of water towards themselves and then 'filtering' the flow for algal food. Operating a filter seems an indiscriminate mode of feeding and one wonders whether one of the consequences of life at low Reynold's number is that zooplankters are of so catholic a taste that they eat everything caught in their 'filters'. If

they do, it seems curious that the algae of the phytoplankton have evolved so many shapes and sizes. Zooplankters must also cope with the consequences of living in a transparent medium. Rapid motion is forbidden by the low Reynold's number and there is nowhere to hide. Their fluid environment also tends to be layered, warm on top and cold underneath. The strategies adopted by zooplankters to deal with these conditions were explored at a conference on the structure of zooplankton communities held at Dartmouth College New Hampshire*.

We now have a better idea of what 'filtering' really means because the process has been watched by high-speed cinematography through a microscope (J. R. Strickler, University of Ottawa; J. J. Gilbert, Dartmouth Col-

Erratum

In the article 'Epithelial cancer, differentiation and vitamin A' (*News and Views* 277, 261; 1979) the wrong illustration was inadvertently included. The correct diagram is given below.



*Held on 21–25 August, 1978.

hunting by sight. There were more confirmatory data to support the validity of this hypothesis, particularly of the kind that correlates cyclo-morphic phenomena with the presence of fish. In addition, it has been nicely shown that the visual acuity of planktivorous fish is of the order expected by the hypothesis so that reducing apparent size is a valid strategy for plankters. There is consensus that predation is, indeed, the selective force behind the cyclomorphosis of the larger cladocerans.

Attention has now shifted to the adaptive responses produced by small invertebrate predators. Some of these are at low Reynold's number like their prey, and have comparatively low success rates. Others, like *Chaoborus*, can cross the Reynold's number divide, make fast capture movements, and wait in ambush on vertical migration paths. The rotifer *Asplanchna*, an animal not much larger than the other rotifers on which it preys, is most successful against victims that are not spiny, suggesting that spines are an adaptation to *Asplanchna* predation. *Heterocope* predation can induce the possession of spines in prey populations rather as fish predation induces cyclomorphosis in Cladocera. There was a general feeling at the conference that small predators may be more important in producing structure in zooplankton communities than competition between herbivores.

With filtering, vertical migration, and cyclomorphosis becoming understood we may claim to be mastering some of the immediate causes of community structure in the plankton. The underlying causes remain obscure. The difficulty in deciding even whether responses involve selection or merely phenotypic plasticity can be gauged by the fact that there still seems uncertainty about what constitutes many planktonic species. Taxonomists hesitate to put names on tropical zooplankters, not feeling sure whether many of these can be fairly assigned to species known from temperate latitudes, and there seems a growing doubt that species from temperate Europe and North America are really the same even though they look extremely similar. A further question is how much of the response to selective pressures such as predation can be genetic when typical populations are asexual clones?

One particular peculiarity was called to mind by several observations from the tropics: there are usually very few species of zooplankton in the open water of a tropical lake, perhaps one or two copepods, one or two cladocerans, and a few rotifers. This comparatively tiny assemblage of herbivores feeds on an array of phytoplankton

Magnetics, climate and eccentricity

from Peter J. Smith

ONE of the most surprising correlations to have been discovered in recent years is that between fluctuations in palaeoclimate and the palaeomagnetic field—surprising because it is difficult to imagine any possible causal connection between an atmospheric phenomenon and motions in the Earth's fluid outer core. Indeed, so astonishing was the geomagnetism-climate relationship that, when its existence was first hinted at, a good many people were inclined not to believe it. But if there are still any sceptics around, it may be presumed that they never saw the report by Wollin *et al.* (*Geophys. Res. Lett.* **4**, 267; 1977) published some 18 months or so ago. For in it Wollin and his colleagues presented data from several deep sea sediment cores, showing a convincing general (anti) correlation between the intensity of core magnetisation and foraminiferal abundance—which means a correlation between warm climatic stages and periods of low geomagnetic field intensity.

They went even further. Harrison (*Earth-Sci. Rev.* **10**, 1; 1974) and Kent and Opdyke (*Eos* **57**, 237; 1976) had already suggested that any relationship between geomagnetic field and climate may be the result of a third phenomenon; and Hays *et al.* (*Science* **194**, 1121; 1976) had linked ice ages with the obliquity and precession of the Earth's orbit. Wollin and his coworkers therefore plotted not only magnetisation and foraminiferal abundance but also the variation in the Earth's orbital eccentricity as deduced by Van Woerkom (in *Climatic Change* (ed. Shapley, H.) Harvard University Press, 1953). A threefold correlation between warm climate, low field intensity and high orbital eccentricity for the past 0.8 Myr was thus revealed, the clear implication being that eccentricity modulates both climate and geomagnetic field.

In view (presumably) of previous scepticism on the climate—

geomagnetism issue, Wollin *et al.* were rather cautious about the reality of the relationship emerging from their data. But if their new results are anything to go by they need not have worried, for they have now demonstrated a similar correlation in a single North Atlantic sediment core spanning no less than the whole of the past 2 million years (*Earth planet. Sci. Lett.* **41**, 395; 1978). This time they used the more recent eccentricity data from Vernekar (*Meteorol. Monograph* **12**, American Meteorological Society, 1972), but the result was the same. Warm climate matches low field matches high eccentricity.

But why? For the time being that is anyone's guess; so Wollin and his colleagues have had a go at it themselves. They begin with the premise that because the density of the Earth's core is greater than that of the near-surface zone, the core-mantle boundary is less elliptical than the Earth's surface. They then argue that, as a result, the torque exerted by solar and lunar gravity on the core is smaller than that on the mantle; so the core tends to precess more slowly than the mantle. Moreover, as the Earth's orbit is eccentric, the solar gravitational field acting on the Earth has an annual variation that increases as the eccentricity increases. Therefore when the eccentricity is greater, the difference between the torques acting on mantle and core is greater, and the tendency of the core and mantle to precess at different rates is enhanced.

Wollin *et al.* then go on to suggest that such an increase in the differential precession will lead to perturbations in the core's convective flow of such a type as to reduce the strength of the geomagnetic dipole and hence reduce the effectiveness of the magnetic shield against corpuscular radiation. Anyone else want to try?

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species that may number 200 or so different kinds. On land, such an array of plants always seems to be under pressure from many different herbivores, and we call on the 'cropping principle' to explain plant diversity. It is an apparent paradox of the zooplankton that natural selection has not provided an array of herbivore species to specialise on the diverse array of

plants. Probably the answer to this paradox lies in the fact that the discriminatory powers of planktonic herbivores, so clearly revealed at this conference, are yet rudimentary compared with those of small terrestrial herbivores. □

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articles

Effect of climatic warming on the West Antarctic ice sheet

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Climatic warming could cause increased melting from Antarctic ice shelves. Continued weakening of the ice shelves in this way would result in the ultimate collapse of most of the West Antarctic ice sheet. For complete removal of the ice shelves collapse of the ice sheet and a 5 m rise in world sea level could occur in <100 yr. More realistically, ice-shelf deterioration is likely to be a rather slow process, and even for a major and sustained warming trend ice-sheet collapse would take several hundred years, with most of the associated rise in sea level occurring during the final century. However, little is known about the glaciers that drain the northern part of the ice sheet. These glaciers have little or no protective fringe of ice shelf and, unless they flow over sufficiently high bedrock sills, they may show a more rapid response to increased temperatures.

MERCER warned recently¹ that increasing levels of atmospheric CO₂ may lead to climatic warming that is sufficiently magnified in high latitudes to cause the demise of the Antarctic ice shelves and the inevitable collapse of the West Antarctic ice sheet, leading to a 5 m rise in world sea level. Although at present, parts of the Ross Ice Shelf seem to be growing thicker², Mercer's warning cannot be dismissed lightly as the West Antarctic ice sheet is undoubtedly buttressed by ice shelves, and these ice shelves are highly susceptible to changes in climate. Mercer suggested² that significant climatic warming may be imminent if not already in progress, and here we try to estimate how rapidly ice-sheet collapse could occur and which parts of the ice sheet are most susceptible. First, however, we stress the need for more sophisticated modelling of climatic response to changing levels of atmospheric CO₂ since there is no general agreement regarding the sign, let alone the magnitude, of the response. Here we shall simply assume that warming, for whatever reason, is sufficient to increase ablation rates from Antarctic ice shelves.

The West Antarctic ice sheet (Fig. 1) rests on land that, for the most part, is below sea level³. Most of the drainage from the ice sheet is into the Ross and Ronne ice shelves, which have a stabilising influence as ice flowing across the grounding line has

to push the ice shelf past its sides and around areas where the ice shelf has locally run aground on seabed shoals to form ice rises. The effect of climatic warming would be to weaken the ice shelves and reduce their buttressing effect so that flow rates from the ice sheet would increase. This would provide negative feedback because the advection of thicker ice from upstream would tend to balance the increased melting rates. In this way collapse of the West Antarctic ice sheet would proceed at a slower rate than would be the case for complete removal of the ice shelves. A full analysis of ice-sheet collapse in these conditions is extremely difficult, but we can estimate maximum retreat rates by making some simplifying assumptions.

Warmer temperatures will weaken ice shelves in two ways—by thinning due to melting at the upper and lower surfaces; and by enhancing existing lines of weakness, such as rifts and bottom crevasses, so that calving rates are increased. Disintegration of an ice shelf by such calving is most likely where the ice shelf is in longitudinal tension. This condition holds over most of an ice-shelf surface except upstream from an ice rise, where longitudinal stresses are strongly compressive and calving is unlikely to occur until the ice rise has disappeared. Once this happens the ice shelf is in longitudinal tension all the way from the grounding line to the ice front and, if accelerated calving does occur, the calving front may recede to the grounding line. In order to incorporate the effects of both increased calving and increased melting within a mathematically tractable model we shall assume that most of the Ross Ice Shelf is instantaneously removed by accelerated calving along existing lines of weakness (Fig. 1). Only the portions of ice shelf that are protected by ice rises remain and these persist until the ice shelf floats free from the seabed shoals beneath the ice rises. We shall restrict our analysis to the two most active ice streams (B and E in Fig. 1) that flow into the Ross Ice Shelf. These ice streams flow along bedrock troughs and they are separated from neighbouring ice streams by ridges or domes of slow-moving ice that rests on bedrock ridges³. Outflow from both ice streams is currently restricted by two ice rises within the Ross Ice Shelf—Crary Ice Rise and Roosevelt Island (Fig. 1). We assume that the ice streams are parallel sided and that, as the grounding line retreats, a floating ice shelf forms between the marginal ice ridges (Fig. 2). The seaward grounding line of these ridges will also retreat, but rather slowly because the ice is probably frozen⁴ to the comparatively shallow bedrock. In our analysis we assume that during ice-sheet collapse the only restrictions to outflow are

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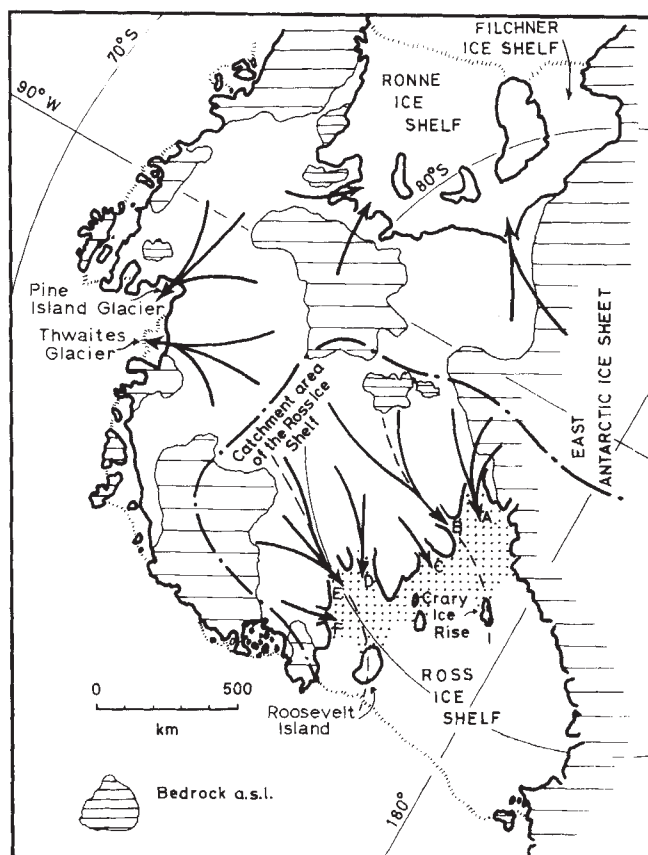


Fig. 1 West Antarctica, showing the areas of bedrock that are above sea level. The arrows represent ice streams that drain the ice sheet. Most of the ice that flows into the Ross Ice Shelf from West Antarctica drains through ice streams B and E. Pine Island Glacier and Thwaites Glacier carry most of the ice that drains northwards. These glaciers seem to be relatively unprotected by bounded or partially grounded ice shelves. Vertical sections taken along the broken lines through ice streams B and E are shown in Fig. 3. In order to calculate maximum ice-sheet retreat rates resulting from climatic warming we assume that most of the Ross Ice Shelf disintegrates very rapidly by calving of icebergs, to leave only the stippled area upstream of the ice rises.

the two ice rises and shear at the sides of the ice shelf that forms within the channel to seaward of the grounding line (Fig. 2). By neglecting the restraining effects of the rest of the Ross Ice Shelf we allow the possibility that accelerated calving may occur, and by assuming instantaneous removal of this portion of the ice shelf we ensure that calculated retreat rates represent reasonable upper limits. We admit that even higher retreat rates can be obtained if we assume instantaneous removal of the entire ice shelf. We have calculated these and we include them in our results, but we stress that they represent limiting values which could never be achieved.

Increased melting will weaken the coupling between the ice rises and the ice shelf, but ice rises will continue to exist and to limit ice-stream flow rates until the ice shelf becomes too thin to run aground. By modelling the form of the ice shelf⁵ for given grounding-line positions, marginal constraints, ablation rates and values of the compressive force F between ice shelf and ice rise, we can find the minimum velocity V across the grounding line necessary for the ice shelf to remain grounded on a given shoal. Thus, there is a relationship between F and V [$V = f(F)$] which holds while the ice rise exists. In addition, equilibrium conditions at the grounding line give an independent relationship⁶ between F and V [$V = g(F)$] in terms of snow accumulation over the ice sheet and of grounding line retreat rate. If the solution of these two equations gives positive values of F and V for a given position of the grounding line then the ice rise

continues to exist and the grounding-line retreat rate can be calculated from V . As $F \rightarrow 0$ the influence of the ice rise diminishes until it becomes ungrounded at $F = 0$. Thereafter we consider two possibilities: instantaneous calving of the remaining ice shelf so that further retreat is unrestricted, or persistence of ice shelf within the ice-stream channel so that retreat is delayed by shear at the ice-shelf sides. Retreat of the grounding line between ice sheet and ice shelf occurs because the thinning effects of creep and ablation are greater than thickening by snow accumulation and the advection of thicker ice from upstream⁶. In order to calculate the rate of retreat we have used available measurements of snowfall over West Antarctica⁷, ice-shelf flow parameters from observations on existing ice shelves⁸, and a value for shear stress between the ice shelf and its sides (0.5 bar) which is consistent with observations of floating ice that is flowing rapidly between parallel sides⁹. The effects of advection are incorporated by adopting a relationship between ice-stream velocity V and the product of ice thickness H and surface slope α : $V = C(H\alpha)^m$, where $m = 3$, and $C (\sim 550 \text{ m}^2 \text{ yr}^{-1})$ is calculated from the present-day values of $V \sim 550 \text{ m yr}^{-1}$ (ref. 10) and $H\alpha \sim 1 \text{ m}$ (ref. 3 and Fig. 3) from near the grounding line of ice stream B. This form of glacier sliding law has some support from theory¹¹, but it is not expected to be generally valid. Here it is used solely to calculate values of α at high ice velocities, and we have chosen the highest probable values of m and C so that we obtain the lowest probable values of surface slope. This means that we probably underestimate the ice advection term and overestimate grounding-line retreat rates. This approximation is clearly a weakness in our model but it cannot be improved until more is known about the dynamics of fast glaciers and ice streams. Meanwhile our approach is useful as it gives an estimate of the probable minimum time for ice-sheet collapse.

Creep thinning at the grounding line increases rapidly with ice thickness¹², and decreases with increasing back pressure due to ice rises and shear between the ice shelf and its sides¹³. The longitudinal profiles³ of ice streams B and E are shown in Fig. 3. Once the grounding line retreats down the bottom slope into the deep basin beneath ice stream B creep thinning is controlled by the large values of ice thickness, and retreat is very rapid even for the model that allows increasing back pressure to be exerted by the ice shelf that is assumed to form seaward. Figure 3 shows grounding-line position during retreat plotted against time for difference rates of melting beneath the ice shelf. In contrast ice stream E has a comparatively horizontal bed so that creep thinning decreases as the grounding line retreats to leave an ever larger ice shelf within the ice-stream channel. In these condi-

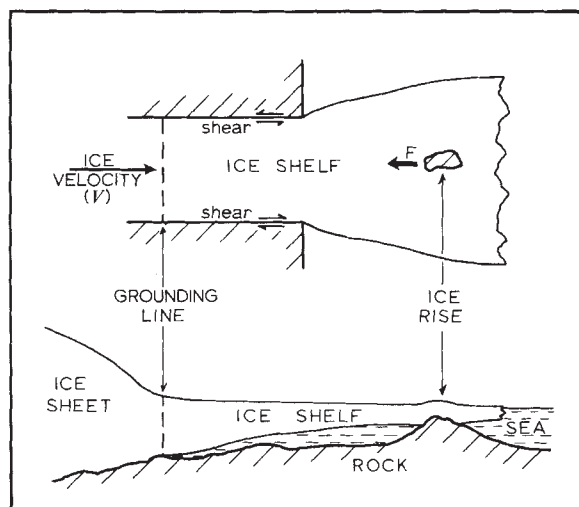


Fig. 2 A retreating ice stream. As the grounding line retreats ice shelf forms between the ridges of slow moving ice that formed the margins of the ice stream. Movement of the ice shelf is restricted by shear past its sides and by the presence of an ice rise where the ice shelf locally has run aground.

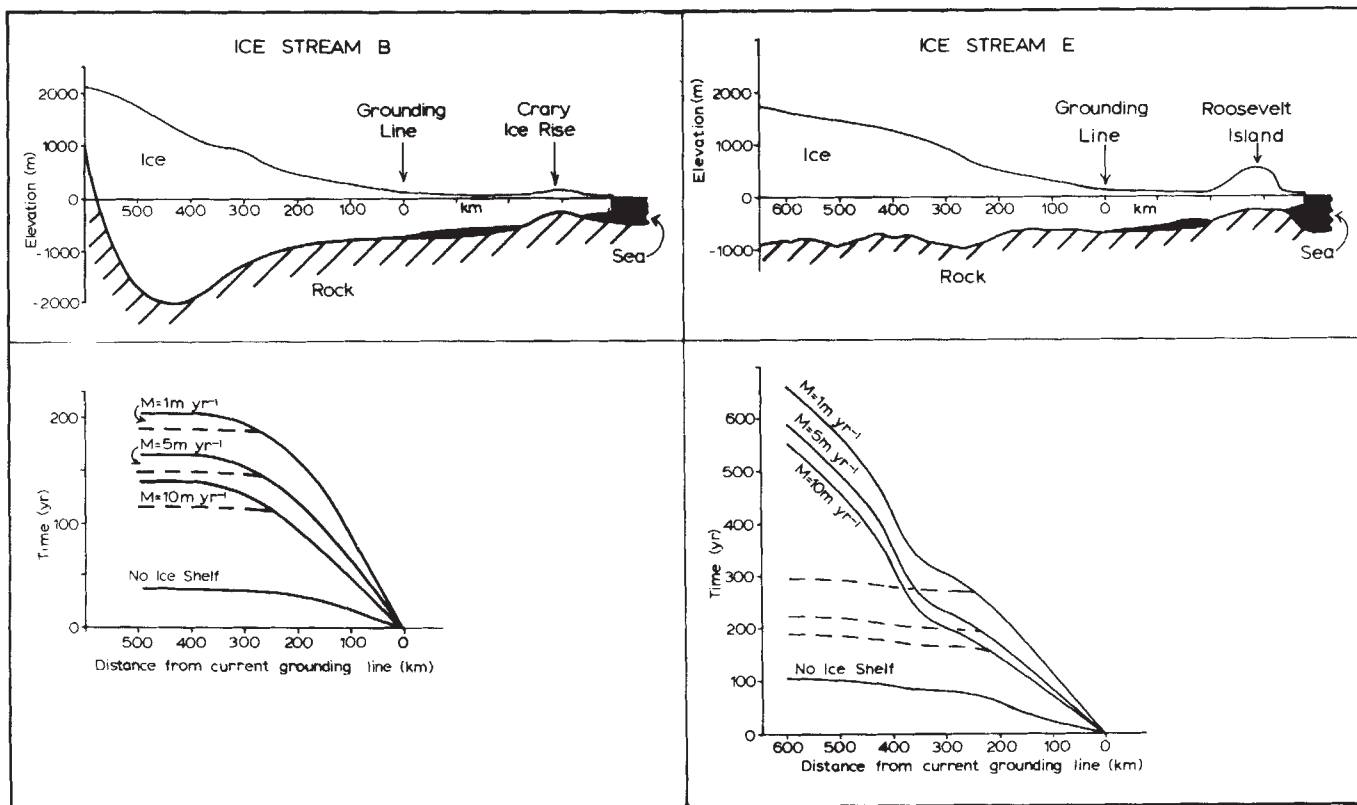


Fig. 3 The longitudinal profiles of ice streams B and E as they are today, and plots of grounding-line position versus time for these ice streams during retreat. Retreat is most rapid if the ice shelf is completely removed, and least rapid for low values of ice-shelf melt rate, M . Once the grounding line reaches the deep basin beneath ice stream B retreat is extremely rapid. In contrast, ice stream E flows over a comparatively horizontal bed and retreat rates are ultimately determined by the rate of collapse of the ice-shelf margins. The broken lines show retreat following complete breakup of the ice shelf as the ice rises collapse.

tions the grounding line may achieve a quasi-equilibrium position once the ice shelf is so large that creep thinning is sufficiently reduced. However, the ice-shelf margins are themselves retreating and the ice-stream grounding line retreats at approximately the same rate (Fig. 3). Changing the melt rates affects only the time taken for the ice rises to collapse. This is because we have not altered the ablation rate at the grounding line, which we have assumed to be 1 m yr^{-1} in all cases. Unless the climate changes dramatically this is a probable maximum for the ice streams. Melting rates beneath ice shelves may be very high^{14,15} and our model allows values up to 10 m yr^{-1} at the ice rise with a linear variation between grounding line and ice rise. Our results underestimate the influence of the ice rises because we have allowed unrestricted lateral creep once the floating ice leaves the ice-stream channels. In reality, neighbouring areas of ice shelf would restrict lateral creep so that vertical thinning would be reduced and the ice rises would survive longer than calculated. Thus, once again, our model overestimates probable retreat rates.

Figure 3 includes plots of grounding-line position against time for retreat with icebergs calving at the grounding line so that there is no ice shelf to restrict outflow. In these conditions collapse of ice streams B and E could occur within 40 and 110 yr respectively, and the rapidly retreating ice stream B would probably capture portions of the catchment areas of neighbouring ice streams to render them more and more unstable. In this way, without the protection of the Ross Ice Shelf, most of the West Antarctic ice sheet could almost completely collapse within 100 yr. However, if we allow ice shelves to persist upstream of the ice rises, retreat is significantly slowed so that total collapse of ice stream B takes between 100 and 200 yr depending on whether ice shelf survives after the ice rises collapse. Equivalent values for ice stream E are 200 and 700 yr but, because of feedback effects, retreat would be accelerated to

correspond more closely to that of ice stream B, so that most of the West Antarctic ice sheet could collapse within ~ 200 yr. However, our assumptions throughout have been biased to give maximum retreat rates and we stress that we have neglected the buttressing effect of most of the present-day Ross Ice Shelf. Currently, the grounding line of ice stream B seems to be advancing into the ice shelf², and in the recent past there may have been widespread grounding and subsequent thickening in the lower reaches of ice stream C (ref. 4). Thus, our estimated collapse rates would apply only after removal of large areas of ice shelf following a major increase in melt rates, probably from the ice/water interface. The shift in oceanographic parameters necessary to effect such an increase would be a rather slow response to climatic change¹⁶, and our calculations take no account of how long this would take. We expect the response time of the oceans to be of the order of hundreds of years¹⁷, so that initial retreat would be far slower than our calculations suggest. Later, as the ocean feels the effect of changing climate and as more of the ice shelf disintegrates, our model would become progressively more applicable. In effect this means that, after the climate starts to change, the first 100–200 km of retreat would take some hundreds of years and then the retreat would follow the pattern shown in Fig. 3. The entire sequence would probably take at least 400 yr, even for a major warming trend of 5°C per century, and most of the collapse and its associated rise in sea level would occur during the final hundred years. An example of such a retreat sequence was the disappearance of the marine-based portion of the Laurentide Ice Sheet from Hudson Bay. The final collapse took $\sim 200\text{ yr}$ ¹⁸, but this was probably the culmination of a retreat sequence that had started considerably earlier.

It is important to consider how relevant these conclusions are to the behaviour of other large ice streams in Antarctica. Available data suggest that the grounding line between West

Antarctica and Ronne Ice Shelf is well protected by large ice rises (Fig. 1) so that probable retreat rates are covered by our analysis. However, the major drainage from the north coast of the West Antarctic ice sheet is through the Thwaites and Pine Island glaciers (Fig. 1), and these terminate in small ice shelves which may or may not be buttressed by ice rises. The stability of these ice streams is dependent on suitably high rock sills near the ice-stream grounding lines. The critical depth (d) below sea level of these sills depends to a large extent on ice-shelf dimensions and on the presence or absence of ice rises, but if the fringing ice shelves are unrestricted then d may be as low as 400 m for each of these glaciers. Pine Island Glacier may flow into a bounded ice shelf about 50 km square and even this comparatively small ice shelf would exert sufficient back pressure on ice flowing across the grounding line to increase d from 400 m to more than 500 m. An increase in ablation rates would be particularly damaging in this area since there is little or no ice-shelf fringe to regulate drainage rates. Little is known about bedrock topography beneath these glaciers but available data suggest that they flow through deep troughs similar to that of ice stream B. If sufficiently high sills do not exist to provide stable grounding-line positions for these glaciers then the northern part of the West Antarctic ice sheet could already be collapsing. Our calculations suggest that, if the seabed at the grounding line of either glacier is 200 m deeper than the equilibrium value, then the grounding line is retreating at 1.5 km yr^{-1} with associated ice velocities of 10 km yr^{-1} . This would be sufficient to raise sea level by approximately 1 mm yr^{-1} . Preliminary estimates of Thwaites Glacier velocities greater than 2 km yr^{-1} have been made by R. Alan of the U.S. Geological Survey from a comparison of satellite imagery with aerial photographs. This is approximately the velocity appropriate to equilibrium with a sill at the critical depth d . If this is the case then Thwaites Glacier would be particularly susceptible to any increase in ablation rates.

We conclude that, if the CO_2 greenhouse effect does cause climatic warming that is magnified in polar regions, we do not expect a major increase in ice-shelf ablation rates until ocean

warming is sufficient to increase melt rates beneath the ice. Even with a major climatic change and high ablation rates collapse of the West Antarctic ice sheet through ice streams that drain into the Ross and Ronne ice shelves would be restricted initially because the ice shelves would continue to regulate drainage rates, and total retreat would probably take more than 400 yr with most of the associated rise in sea level occurring during the final century. However, much of the ice-sheet drainage is to the north through glaciers that may be unprotected by ice shelves. Unless these glaciers flow over sufficiently high bedrock sills they may be in a very precarious state of equilibrium or they may already be collapsing, and significant warming could rapidly accelerate their collapse. In order to improve our assessment of the stability of these glaciers a detailed survey of their basal topography and velocity measurements on their floating tongues are urgently needed.

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Observations from the IDA network of attenuation and splitting during a recent earthquake

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The project for the International Deployment of Accelerometers (IDA) is intended to provide very low frequency seismic data for the study of Earth structure and source mechanisms. This article outlines preliminary results on attenuation and splitting derived from IDA recordings of a recent large earthquake.

ON 19 August 1977 a very large earthquake occurred about 200 km south west of the island of Sumbawa, Indonesia. The USNEIS hypocentral coordinates are 11.13° S , 118.40° E and 20 km depth. The origin time was 06:08:52.6 GMT. A body-wave magnitude of 7.75 has been assigned by the U.S.G.S.

Stewart¹ has estimated the moment to be 10^{29} dyn cm (10^{22} Nm). As this seems to have been the most energetic earthquake since the 1965 Rat Island event it is ideal for the study of very long period free oscillations. The data we have used for this purpose are from the project for the International Deployment of Accelerometers (IDA)². The six stations of the IDA network that produced good records for this event were located at Fairbanks, Alaska (CMO); Naña, Peru (NNA); Sutherland, Republic of South Africa (SUR); Halifax, Nova Scotia, Canada (HAL); Brasilia, Brazil (BDF); and Rarotonga, Cook Islands (RAR). For the analysis presented here record lengths up to 526 h were used. Part of a typical record (RAR) is shown in Fig. 1. The first 4 h of the recording have been discarded since the large signals saturated the system. Before computing the spectrum, the larger bodily tides have been removed by a least squares fitting procedure and aftershocks have been excised and replaced by linear interpolation.

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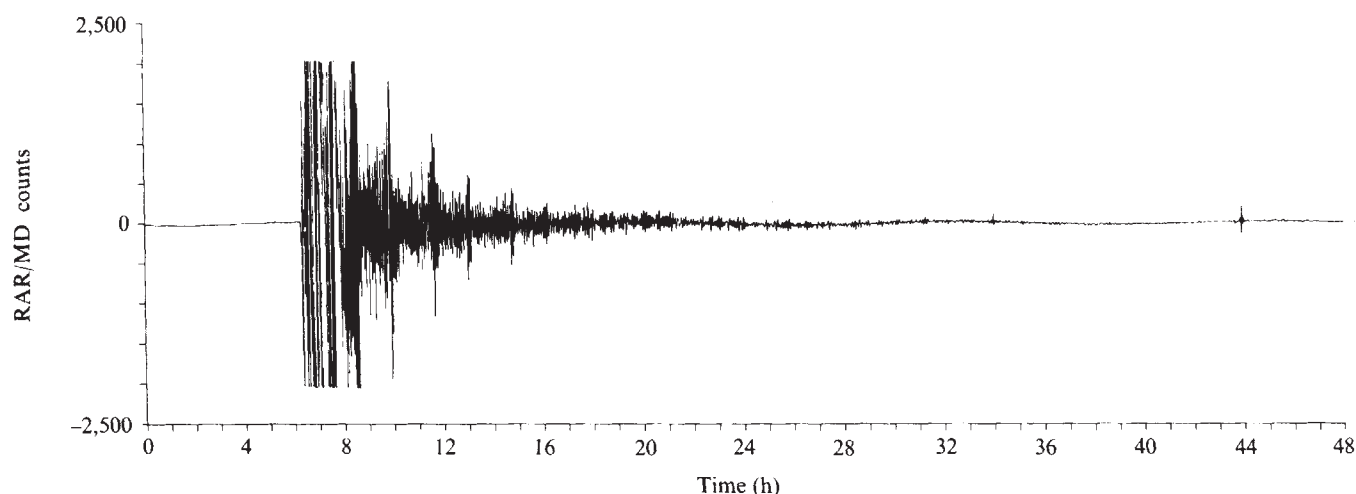


Fig. 1 A 48-h record from IDA station RAR, typical of the data from the Sumbawa event. Note that the instrument was saturated during the first 4 h of the event (through R3). Also Raleigh packets are clearly visible out to R8.

Figure 2 shows the Fourier power spectrum of 155 h of the BDF record beginning 25 h after the event. Clearly present in the spectrum are the fundamental spheroidal multiplets ${}_0S_2$ – ${}_0S_{21}$, several overtones, and the radial modes ${}_0S_0$ and ${}_1S_0$. The low order multiplets ${}_0S_2$ – ${}_0S_4$ are obviously split. The quality of the data encouraged us to examine them in more detail and we present here preliminary measurements of frequency splitting, attenuation, and degenerate multiplet frequencies.

Splitting

A multiplet ${}_nS_l$ has $2l+1$ singlet lines. Spherical symmetry causes these lines to have the same degenerate frequency. Deviations from spherical symmetry such as rotation, ellipticity, and lateral variations remove the degeneracy, wholly or partially, and cause the multiplet to be split³ as may be clearly seen in Fig. 2 for the modes ${}_0S_2$, ${}_0S_3$, and ${}_0S_4$. To investigate this splitting, we can interpolate the discrete Fourier transform (DFT) to a frequency spacing finer than

$$\Delta f = 2\pi/T$$

where T is the length of the time series, by numerically evaluating the trigonometric series approximation to the Fourier integral. A more complete discussion is given elsewhere⁴.

Figure 3 shows spectra for ${}_0S_3$ from six individual records. If we count the individual peaks in Fig. 3 we find more than the seven we know must be there. A simple calculation shows that the width (half width at half power) of each singlet is about $9 \times 10^{-6} \text{ rad s}^{-1}$ (due both to attenuation and finite record length). Theoretically, the average line spacing for this multiplet should be $1.36 \times 10^{-5} \text{ rad s}^{-1}$ (ref. 5). Clearly, the problem is interference between the strong and weak singlets.

There are three ways we know of to resolve the singlets. The first is to increase the record length. Doubling the time series length would narrow the lines to $5.5 \times 10^{-6} \text{ rad s}^{-1}$, but the low amplitude singlets would be overwhelmed by noise. The second method is analytical continuation⁴. This would narrow the lines to $5 \times 10^{-6} \text{ rad s}^{-1}$, but would also decrease the signal-to-noise ratio (S/N) unacceptably. The third method is stacking⁶, which enhances the S/N and tends to cancel adjacent singlets. Thus, the only practicable method requires the use of the stations as an array.

At frequencies below 1 mHz we expect that the dominant perturbation from spherical symmetry will be the Coriolis force. Thus, to zeroth order in perturbation theory, the radial (vertical) component of motion of a singlet is proportional to $Y_l^m(\theta, \psi)$, the complex spherical harmonic, in geographical (north polar) coordinates. Each singlet, except $m=0$, is a travelling wave⁷.

Let $U(\omega, \theta_j, \psi_j)$ be the spectrum of the vertical component at the j^{th} station. For an isolated multiplet ${}_nS_l$

$$U(\omega, \theta_j, \psi_j) = \sum_{m=-l}^l {}_nA_l^m Y_l^m(\theta_j, \psi_j) {}_nC_l^m(\omega)$$

where ${}_nA_l^m$ is the excitation coefficient and ${}_nC_l^m$ is the singlet spectrum. Since the spherical harmonics are orthogonal we form the stacked spectrum

$${}_nS_l^m(\omega) = \sum_j \bar{Y}_l^m(\theta_j, \psi_j) U(\omega, \theta_j, \psi_j).$$

where the overscore means complex conjugation. For an isolated multiplet we obtain

$${}_nS_l^m(\omega) = \sum_j \bar{Y}_l^m(\theta_j, \psi_j) \sum_{m'=-l}^l {}_nA_l^{m'} Y_l^{m'}(\theta_j, \psi_j) {}_nC_l^{m'}(\omega)$$

Dropping the n, l indices we have

$$S_m = \sum_{m'=-l}^l A_{m'} \left[\sum_j \bar{Y}_l^m(\theta_j, \psi_j) Y_l^{m'}(\theta_j, \psi_j) \right] C_{m'}(\omega)$$

For a large number of uniformly spaced stations the term in brackets approaches a surface integral which equals the Kronecker $\delta_{mm'}$ and $S_m = A_m C_m(\omega)$. Even for a small number of stations one singlet is added always in phase while the others are not and therefore tend to cancel.

Operationally, we must render our six seismograms directly comparable. Therefore, we have removed the instrument phase and normalised the instrument amplitude to a standard response⁸. Finally, we have selected a common time window leaving us with six 155-h records beginning 25 h after the event. Gilbert and Dziewonski⁶ show how to avoid discarding data as we have done, but their method involves assumptions about attenuation which we are unwilling to make.

We have applied the spherical harmonic stacking procedure to the multiplet ${}_0S_3$ and representative results are shown in Fig. 4 for $m = -3, 0, 3$. The spherical harmonic stacking procedure has worked well. Using the moment ratio method⁴ we have measured all $2l+1$ (that is, seven) singlet frequencies ω_m . This is the first time that a split multiplet has been completely resolved.

Let ω_d be the degenerate eigenfrequency in the absence of rotation and ellipticity of figure. (We ignore other perturbations.) The relation between ω_m and ω_d is

$$\omega_m = \omega_d(1 + a + mb + m^2c)$$

The perturbation parameter b arises from the first order effect of Coriolis force. The perturbation parameter c arises from ellipticity (c_e), the non-radial part of the rotation potential (c_n), and

the second order effect of Coriolis force (c_2)

$$c = c_e + c_n + c_2$$

The perturbation parameter a has, in addition,

$$a = a_e + a_n + a_2 + a_r$$

a term a_r from the radial part of the rotation potential. We have

$$a_s + \frac{l(l+1)}{3} c_s = 0 \quad \text{for } s = e \quad \text{and } n$$

Thus $\bar{\omega}$ the mean of the $(2l+1)\omega_m$ is

$$\begin{aligned} \bar{\omega} &= \frac{1}{2l+1} \sum_{m=-l}^l \omega_m \\ &= \omega_d (1 + a_2 + a_r + \frac{l(l+1)}{3} c_2) \\ &= \omega_d (1 + p) \end{aligned}$$

From our observations of ω_m we can obtain $\bar{\omega}$, but to obtain ω_d we must have p , a functional of the Earth's structure, given the rate of rotation. Tables of a_s , b , and c_s have been prepared⁵. The value of p changes very little from one acceptable Earth model to another. We are probably justified, at this state of our knowledge, in using the tables to infer ω_d from $\bar{\omega}$. For ${}_0S_3$ we have computed ω_d and $\omega_m^{(com)}$ from $\bar{\omega}$ and tabulated values of a_s , b , and c_s . The comparison is shown in Table 1.

The differences between $\omega_m^{(com)}$ and $\omega_m^{(obs)}$ have been used to compute a (relative) standard error of the mean (s.e.m.) of $\bar{\omega}$ of 1.7×10^{-4} . The perturbation parameter p seems to vary by less than 20% among the acceptable Earth models leading to a

relative change in ω_d of less than 8.6×10^{-6} . Thus the relative error in $\bar{\omega}$ can safely be assigned to ω_d as well.

We have also applied the spherical harmonic stacking procedure to the ${}_0S_2$ multiplet. Cancellation is not as good as for ${}_0S_3$ but the frequency spacing between singlets is greater. Again we have completely resolved the multiplet, revealing two of the five singlets which were not visible in the original data. We have computed $\bar{\omega}$, ω_d , and $\omega_m^{(com)}$ (as for ${}_0S_3$) and displayed them along with $\omega_m^{(obs)}$ in Table 2. The relative error in $\bar{\omega}$ is 5.0×10^{-5} . A 20% change in p leads to a relative change in ω_d of 2.1×10^{-5} . Thus, it is reasonable to assign a relative error of 5.0×10^{-5} to ω_d .

The agreement between observed and computed singlet frequencies in Tables 1 and 2 is good evidence that the theoretical splitting formula⁵ is correct and that model values of a , b and c are correctly computed and are close to the truth. From the data in Tables 1 and 2 we can determine a best fitting quadratic in m

$$\omega_m = \omega_0 + mf + m^2g$$

by the method of least squares, where

$$\omega_0 = \omega_d(1+a); \quad f = \omega_d b; \quad g = \omega_d c$$

The results for ${}_0S_3$ are

$$\begin{aligned} \omega_0 &= 2.94523 \times 10^{-3} \quad s^{-1} \\ f &= 1.378 \times 10^{-5} \quad s^{-1} \pm 0.78\% \\ g &= -4.27 \times 10^{-7} \quad s^{-1} \pm 13\% \end{aligned}$$

where the errors are computed assuming that each $\omega_m^{(obs)}$ is equally accurate. For comparison, model values are

$$\begin{aligned} f^{(com)} &= 1.360 \times 10^{-5} \quad s^{-1} \\ g^{(com)} &= -3.47 \times 10^{-7} \quad s^{-1} \end{aligned}$$

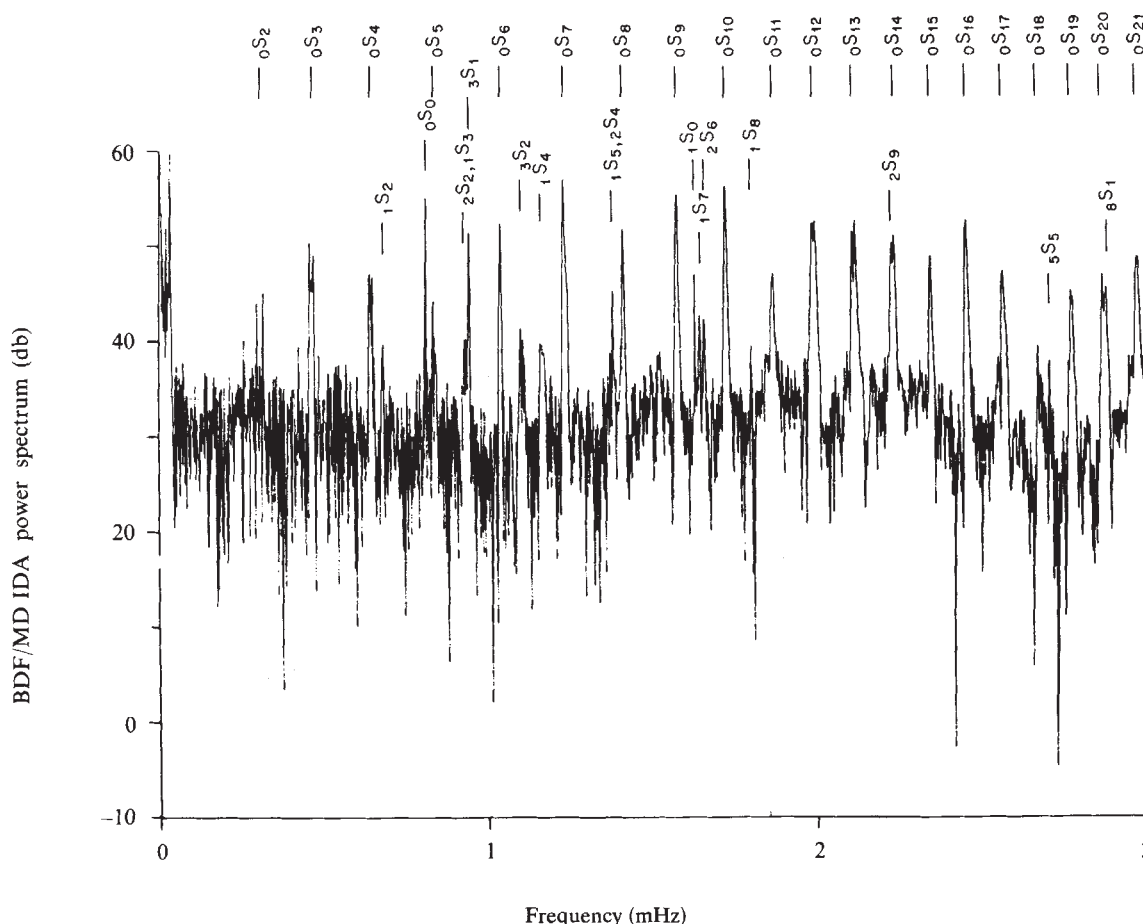


Fig. 2 A log power spectrum of 155 h of record from station BDF beginning 25 h after the event. Note particularly the good excitation of ${}_0S_0$, and many overtones as well as the complexity (due to splitting) of the lowest order fundamental modes: ${}_0S_2$, ${}_0S_3$, and ${}_0S_4$.

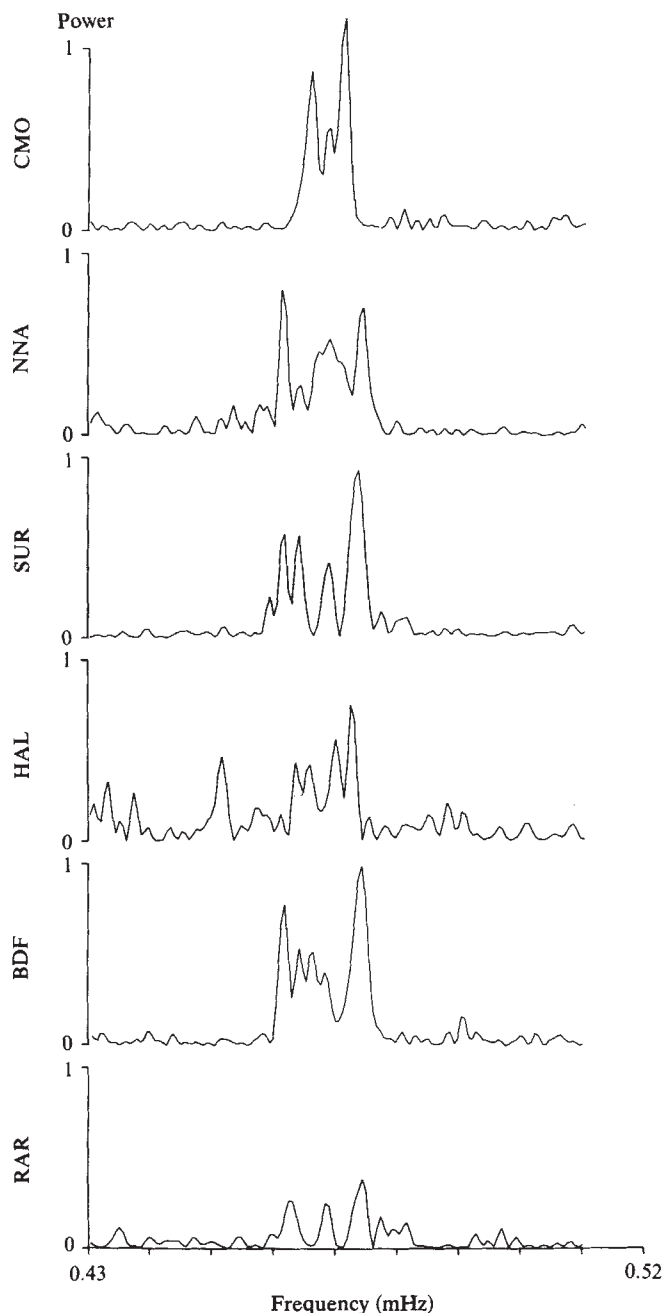


Fig. 3 Interpolated linear power spectra (arbitrary units) of the fundamental mode ${}_0S_3$ for each of the six stations used. In each case, 155 h of record beginning 25 h after the event were used.

The difference between computed and observed values of f is 1.7σ or 1.3% . For g the difference is 1.8σ or 23% .

The results for ${}_0S_2$ are

$$\begin{aligned}\omega_0 &= 1.94445 \times 10^{-3} \text{ s}^{-1} \\ f &= 2.9015 \times 10^{-5} \text{ s}^{-1} \pm 0.46\% \\ g &= -3.407 \times 10^{-7} \text{ s}^{-1} \pm 31\%\end{aligned}$$

The computed⁵ values of f and g are

$$\begin{aligned}f^{(\text{com})} &= 2.8973 \times 10^{-5} \text{ s}^{-1} \\ g^{(\text{com})} &= -5.192 \times 10^{-7} \text{ s}^{-1}\end{aligned}$$

The difference between computed and observed values is 0.3σ or 0.14% for f and 1.7σ or 52% for g .

The computed and observed values of f and g are not significantly different at the 2σ level. However, at the 1σ level,

only f for ${}_0S_2$ shows no significant difference. If further research supports the existence of significant differences between observed and computed values of splitting parameters, then the observed values represent new constraints on models of the structure of the Earth. For example, the Fréchet derivative of f with respect to structure is a functional only of the density distribution. Presumably, if one could observe numerous Coriolis splitting parameters, f , one could obtain improved constraints on the density distribution within the Earth.

Table 1 The singlets of ${}_0S_3$

m	$\omega_m^{(\text{obs})} \times 10^3 \text{ s}^{-1}$	$\omega_m^{(\text{com})} \times 10^3 \text{ s}^{-1}$
-3	2.90076	2.90114
-2	2.91590	2.91628
-1	2.92903	2.93085
0	2.94575	2.94474
1	2.95881	2.95806
2	2.97301	2.97075
3	2.98137	2.98275
$\bar{\omega} = 2.94352 \times 10^{-3} \text{ s}^{-1}$		$p = 4.3 \times 10^{-5}$
$\omega_d = 2.94339 \times 10^{-3} \text{ s}^{-1}$		s.e.m. = $5.0 \times 10^{-7} \text{ s}^{-1}$

Attenuation

There are surprisingly few really precise data concerning the anelasticity (or Q) of the Earth. While we plan to measure the Q values of all visible modes in our Indonesian data set, for this preliminary report we have decided to concentrate on two unusually interesting modes: ${}_0S_0$ and ${}_1S_0$.

The radial ${}_nS_0$ modes store strain energy primarily in compression, rather than shear⁹, and have large Q values. The modes ${}_0S_0$ and ${}_1S_0$ in particular have less shear energy than the other radial modes and the measurement of their extremely high Q values has proved to be elusive. Our data set affords us the unique opportunity not only to measure these Q values, but to explore the reproducibility of the results. Our procedure, therefore, will be to examine the six records separately for consistency, and derive our final Q value from the spherical harmonic stack. We measure α_0 where $\alpha_0 = \omega_0/2Q$.

Because of the high Q values of these modes the width of the peak due to record length is large compared with the width due to attenuation for the record length we have. In order to use a Q estimator based on measuring width it is necessary to make the width due to attenuation comparable with the width due to record length (they are equal at $0.885 Q$ cycles). Conservatively we usually use $1.5Q$ cycles or over 2,000 h in the case of ${}_0S_0$. Even at that we would have lost only 3 db over the maximum attainable S/N (at about 560 h according to Buland and Gilbert⁴). This means that such a measurement would be possible, but we would need to edit another 1,500 h of data on each station. For the data set at hand our experiments have shown that a simple time lapse method works as well as anything. We measure the integrated power between half power points in windows lagged so as to have no overlap.

This is a delicate task. To enhance our statistics we need as many windows as possible. But if each window is too short the signal will be overcome by noise. Further, the total time series length T must be long enough so that the mode has decayed somewhat to gain significance. But, if T is too large the latter windows again will have an unacceptable S/N .

Table 2 The singlets of ${}_0S_2$

m	$\omega_m^{(\text{obs})} \times 10^3 \text{ s}^{-1}$	$\omega_m^{(\text{com})} \times 10^3 \text{ s}^{-1}$
-2	1.88502	1.88489
-1	1.91511	1.91518
0	1.94458	1.94471
1	1.97292	1.97317
2	2.00119	2.00082
$\bar{\omega} = 1.94376 \times 10^{-3} \text{ s}^{-1}$		$p = -1.04 \times 10^{-4}$
$\omega_d = 1.94396 \times 10^{-3} \text{ s}^{-1}$		s.e.m. = $9.8 \times 10^{-8} \text{ s}^{-1}$

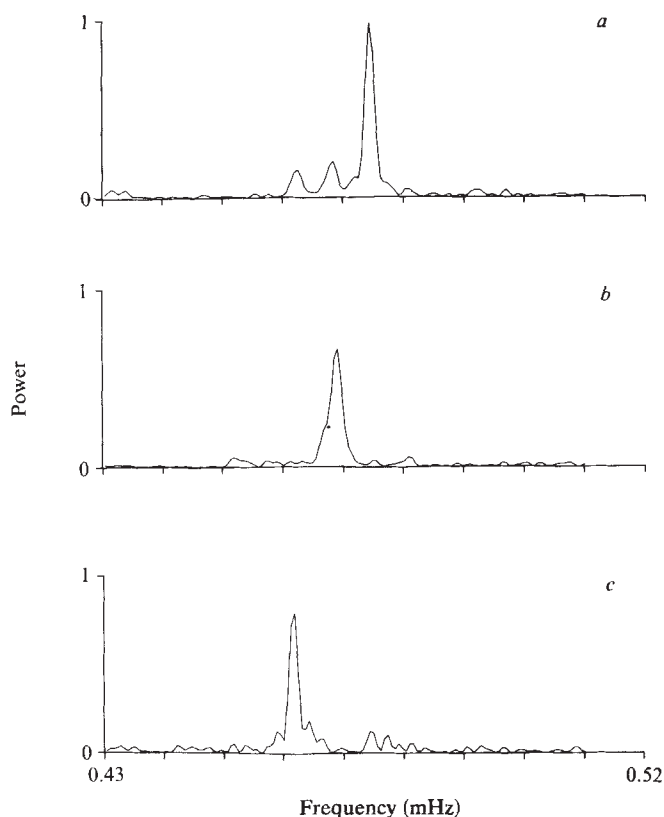


Fig. 4 Interpolated linear power spectra (arbitrary units) of stacks for a , ${}_0S_3^{+3}$; b , ${}_0S_3^0$; c , ${}_0S_3^{-3}$. All seven ${}_0S_3^m$ singlets were resolved.

As a result of these trade offs, we have chosen for ${}_1S_0$ 10^5 -s windows in a 150-h series and for ${}_0S_0$ 2×10^5 -s windows in as long a series as we can get. Therefore, for ${}_1S_0$ we may work with the six 155-h records described above. But for ${}_0S_0$ we will have to do something more elaborate for a stack, as not all the available records are of sufficient length and some of the records have large data gaps. Because of these gaps a straightforward deconvolution of the series to remove the instrument response would distort good data points adjacent to the gaps. The procedure adopted to avoid this was to correct for instrument response at only one frequency by multiplication of the time series by a complex number that is the reciprocal of the instrument response at the desired frequency. Then time series were averaged point by point with data gaps not included in the average, to form a 575-h stack with few data gaps.

For ${}_1S_0$ we used five windows. The Q results for individual records scatter approximately 50% about their mean with relative errors of the least squares fit ranging between 13 and 63%. Our final value is $Q = 2,360 \pm 15\%$, obtained from a 150-h stack. Our final error estimate is the maximum likelihood value derived from the single station measurements. On the basis of the individual record Q values, the final error bound seems to be rather small (it is based on only six data), but not totally unreasonable.

For ${}_0S_0$, the five individual records are of various lengths yielding up to fifteen 10^5 -s windows (avoiding gaps). Individual record Q values scatter as much as 80% about their mean with relative errors of the least squares fit ranging between 13 and 105%. For the final (stack) result we used six 2×10^5 -s windows (to boost S/N). The result is: $Q = 4,100 \pm 26\%$.

Dahlen¹⁰ has extended the work of Munk and Hasselmann¹¹ on the expected quality of spectral parameters measured in the presence of noise. He considered the linear, least-squares estimation of the (complex) frequency of a decaying sinusoid and obtained expressions for the variance

$$\langle \delta \omega_0^2 \rangle = \langle \delta \alpha_0^2 \rangle = \frac{R(2\alpha_0 T)}{T^2(S/N)}$$

The function $R(x)$ is well approximated by $R(x) = 2(x^2 + 9)^{1/2}$. S/N is the ratio of peak signal to average noise in the power spectrum and T is record length.

Let $\sigma_D^2 = \langle \delta \omega_0^2 \rangle = \langle \delta \alpha_0^2 \rangle$. Our result for ${}_1S_0$ gives

$$\sigma_D = 5.0 \times 10^{-7} \text{ s}^{-1}; \quad \sigma_D/\alpha_0 = 23\%$$

for $S/N = 20$ db in the radial stack. The assigned relative error of 15% seems reasonable. In the case of ${}_0S_0$ we have

$$\sigma_D = 5.4 \times 10^{-8}; \quad \sigma_D/\alpha_0 = 9\%$$

for $S/N = 28$ db in the radial stack. Again, the assigned relative error of 26% seems reasonable.

Frequencies

The observations of all singlets in the multiplets ${}_0S_2$ and ${}_0S_3$ allowed us to infer the gross Earth data, ω_d , for these modes with greatly improved precision:

$$\omega({}_0S_2)_d = 1.94396 \times 10^{-3} \pm 9.8 \times 10^{-8} \text{ s}^{-1}$$

$$\omega({}_0S_3)_d = 2.94339 \times 10^{-3} \pm 5.0 \times 10^{-7} \text{ s}^{-1}$$

From the six individual (155-h) records we have measured frequencies for the radial modes ${}_0S_0$ and ${}_1S_0$ with the following results and standard errors of the mean:

$$\omega({}_0S_0) = 5.11850 \times 10^{-3} \pm 1.5 \times 10^{-7} \text{ s}^{-1}$$

$$\omega({}_1S_0) = 1.02532 \times 10^{-2} \pm 1.0 \times 10^{-6} \text{ s}^{-1}$$

As $l=0$ there is only one singlet for each of these modes. However, a computed correction must be made to find ω_d as above. The results are:

$$\omega({}_0S_0)_d = 5.11679 \times 10^{-3} \pm 1.5 \times 10^{-7} \text{ s}^{-1}$$

$$\omega({}_1S_0)_d = 1.02498 \times 10^{-2} \pm 1.0 \times 10^{-6} \text{ s}^{-1}$$

The standard errors assigned to the radial modes are in agreement with the criterion of Dahlen. So are those of ${}_0S_2$ and ${}_0S_3$ provided we take into account the number of singlets in each multiplet and accept theoretical values for α_0 . It would be more satisfactory to use observed values of α_0 and we intend to obtain them.

Discussion

The IDA network has given us the capability to refine our studies of splitting and attenuation, and to improve significantly the resolvability of spectral parameters in the low frequency seismic band. At the time of the Indonesian earthquake there were nine IDA stations, six of which provided the records used in this article. Now there are 14 stations with an additional three scheduled for installation within a year. With an enlarged network we should be able to resolve multiplets of higher order, and improve the accuracy of our measurements of ω_0 and α_0 . Improved measurements will lead to greatly improved models of attenuation and laterally heterogeneous structure of the Earth.

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letters

Evidence of a disrupted pulsar binary system

THIS letter demonstrates that the pulsars PSR1952+29 and PSR1929+10 may have originated in a binary system which was disrupted by a supernova explosion less than a million years ago. If further confirmed by improved observations, this result provides an evolutionary link between the separate phenomena of X-ray binary systems and radio pulsars.

PSR1952+29 has a characteristic age of 3,600 Myr as measured by the usual ratio $\tau = P/2\dot{P}$ (where P is the pulsar period and $\dot{P}$ its time derivative). This is the largest known value. PSR1929+10 has a characteristic age of 3.1 Myr, a more typical value. The characteristic age of a pulsar is generally regarded as an overestimate of its true age, and independent estimates¹ based on kinematic arguments suggest that pulsars have a true average age of 2 Myr.

Recent results² have given the distance to PSR1952+29 as 240 pc, and its closeness to Earth has made it possible to detect its proper motion (200 km s^{-1}) in both magnitude and direction. Gullahorn and Rankin³ report this together with similar measurements of PSR1929+10 and six other pulsars. PSR1929+10, whose galactic coordinates are about 20° from PSR1952+29, is estimated to be at a distance of 110 pc and to have a proper motion of 85 km s^{-1} . Both pulsars lie close to the galactic plane (their galactic coordinates are respectively $l^{\text{II}} = 47.4^\circ$, $b^{\text{II}} = -3.9^\circ$ and $l^{\text{II}} = 65.9^\circ$, $b^{\text{II}} = 0.8^\circ$), with PSR1952+29 actually having a small velocity component into the plane. The velocity vectors of both pulsars point away from a region of the sky from which they can both conceivably have come within a pulsar lifetime. This leads one to suspect that they began their lives as pulsars in a common event. To test this hypothesis it is possible in principle to determine the coordinates of the coincidence from the coordinates and transverse velocities of the pulsars, then to check that both pulsars were at this point at the same time. In practice, errors in the distances and coordinate velocities of the pulsars will mean that the coincidence is never exact. Here we adopt the procedure of choosing possible galactic coordinates for the coincidence (the best fit was given by $l_0^{\text{II}} = 32^\circ$, $b_0^{\text{II}} = 3^\circ$) and computing the velocity which the pulsars would have at their present coordinates if they had started simultaneously from this point. Writing the velocities of PSR1929+10 and PSR1952+29 in galactic coordinates as (v_{l_1}, v_{b_1}) and (v_{l_2}, v_{b_2}) respectively, we obtain the fit

$$\begin{aligned} v_{l_1} &= 80 \text{ km s}^{-1} (78) & v_{b_1} &= -35 \text{ km s}^{-1} (-30) \\ v_{l_2} &= 167 \text{ km s}^{-1} (198) & v_{b_2} &= -12 \text{ km s}^{-1} (-39) \end{aligned}$$

The observed³ values being given in parentheses. The computed values lie within the error bars of the observations. For this calculation it is assumed that the pulsars have moved in straight lines in space, neglecting the gravitational force of the Galaxy (which is a second-order effect for such fast-moving objects). If the two pulsars were coincident at a distance r_0 at a time T ago, then the above fit of their present projected motions on the celestial sphere is given by taking the ratio r_0/T as 300 km s^{-1} . In the absence of measured radial velocities, r_0 and T cannot be determined independently from the transverse motions. If, however, we impose the condition that the radial and transverse

velocity components are comparable, then a possible choice of parameters is $T = 600,000 \text{ yr}$, $r_0 = 180 \text{ pc}$, which yields $v_{r_1} = -106 \text{ km s}^{-1}$, $v_{r_2} = +152 \text{ km s}^{-1}$.

Most supernova remnants have estimated ages $< 0.1 \text{ Myr}$ (ref. 4). It is usually assumed that after this time they merge with the surrounding interstellar medium⁵. The remnant of the supernova taking place 0.6 Myr ago would now, if detectable at all, be a large diffuse area over 30 pc across⁶. At a distance of 180 pc this would have a diameter of at least 10° . Radio maps⁷ do not reveal any such feature centred on the suggested coincidence. However, it is possible to discern a roughly circular feature with a diameter about 7° centred at $l^{\text{II}} = 33^\circ$, $b^{\text{II}} = 12.5^\circ$. Although fresh data on proper motions could bring about a readjustment of the point of coincidence, an association with this feature must be regarded as highly tentative.

Nevertheless, on the basis of the calculations here it seems possible to suppose that pulsars PSR1929+10 and PSR1952+29 emerged from a binary system which was disrupted by an explosive event $\sim 0.6 \text{ Myr}$ ago. As it has the younger characteristic age, one may reasonably assume that PSR1929+10 is the neutron star which was formed in the explosion. Before this, PSR1952+29 will have existed as a neutron star in orbit around a more massive companion which eventually gave birth to PSR1929+10. If confirmed by further measurements, this result provides evidence for van den Heuvel's account⁸ of the final phase of binary evolution and supports the suggestion⁹⁻¹² that pulsars originate in close binary systems.

Without going into the details of close binary evolution here, we can imagine that the binary experience had two important effects on the neutron star which became PSR1952+29. First, having itself been formed in a supernova explosion between 1 and 10 Myr previously⁸, its magnetic field would have had time to decay from its original strength before it was released as a single pulsar. Making the conventional assumption that a pulsar's magnetic field is proportional to $(P\dot{P})^{1/2}$, we obtain a value of $3 \times 10^{10} \text{ G}$ for PSR1952+29, compared with $5.2 \times 10^{11} \text{ G}$ for PSR1929+10 and $3.8 \times 10^{12} \text{ G}$ for the recently-born Crab pulsar. Second, if PSR1952+29 has passed through a spin-up phase (as an X-ray pulsar), then it will have accreted angular momentum from its companion. Its present low braking rate (presumably the consequence of a low magnetic field) implies that its period (0.43 s) has not changed significantly since the break-up of the binary system, but before this both considerable spin-up and spin-down will probably have occurred¹².

A histogram¹³ of the characteristic ages of the 89 pulsars with measured $\dot{P}$ reveals that while 68 of them (including PSR1929+10) belong to a convincing power-law distribution tailing off at 20 Myr, an additional 19 pulsars (including PSR1952+29) do not fit this pattern, having anomalous characteristic ages ranging from 22 to 3,600 Myr. The result here strongly implies that the 'anomalous' pulsars have spent a significant part of their lives as neutron stars in binary systems. The existence of two populations of pulsars arising in this way has been suggested by several authors¹⁰⁻¹². Here we emphasize that the magnetic field will be the factor which distinguishes between them. Precisely this classification of pulsars was made by Helfand and Tademaru¹⁰.

As the experience of PSR1952+29 is not likely to be unique,

Table 1 Examples of possibly 'paired' pulsars

<i>S</i> (pc)	Pair	<i>d</i> (kpc)	<i>P</i> (s)	τ (Myr)
7	1700-18	0.2	0.80	?
	1706-16	0.2	0.65	1.6
18	0809+74	0.2	1.29	128
	0904+77	?	1.59	?
52	1604-00	0.4	0.42	22
	1642-03	0.2	0.39	3.4
91	2106+44	0.8	0.42	66
	2021+51	0.8	0.53	2.7
100	2324+60	0.8	0.23	10
	2223+65	0.7	0.68	1.1
140	1426-66	2.4	0.79	?
	1449-64	2.6	0.18	?
213	1717-29	1.6	0.62	12
	1730-22	1.6	0.87	?
250	0138+59	3.0	1.22	97
	0105+65	1.0	1.28	1.6
300	2305+55	1.6	0.46	75
	2217+47	1.6	0.54	3.1
450	1819-22	5.7	1.87	49
	1754-24	6.3	0.23	?

S is the approximate transverse separation of the pairs; *d*, distance to each pulsar; *P*, period, and τ the characteristic age (data from ref. 13).

it will be of interest to investigate the origins of the remaining 18 anomalous pulsars to see if they have previously unrecognised companion pulsars with whom they once formed binary systems. Some examples of possible 'pairs' are given below in order of increasing transverse separation. Since a pulsar with velocity 200 km s^{-1} travels 200 pc in 1 Myr it is extremely difficult to recognise pairs without accurate velocity vectors, and this list is little more than informed guesswork. The pairs have been chosen on the basis that both pulsars are at roughly the same distance from Earth and are 'reachable' from one another. Assuming the pulsars in each pair have once formed a binary system we have given them in expected order of formation as neutron stars.

The pair PSR1700-18 and PSR1706-16 seems interesting because of their closeness to one another and, as elsewhere on the list, one would at least like to see the question-mark eradicated. Both PSR0809+74 and PSR0904+77 are to be found in a high latitude 'hole' in the galactic HI distribution¹⁴. The association of the 'hole' (presumably a supernova remnant) with PSR0809+74 has been regarded as suspect on account of the latter's large apparent age. It could be that PSR0904+77 is the missing younger companion. A further list of possible pairs, chosen on a similar basis, is given in ref. 9.

Finally, one should consider the future of PSR1952+29 and PSR1929+10. The characteristic age of the latter shows that it is slowing down on a timescale roughly comparable with pulsar lifetimes and it will presumably switch off when larger values of *P* are reached. This argument is not, however, available for PSR1952+29. Its emission may fade through the decay of its already low magnetic field, but the lifetimes of this and the other anomalous pulsars need not bear any relation to the typical lifetimes of their former companions.

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Brightest members of clusters of galaxies

THERE are two opposing interpretations of the small dispersion $\sigma_1 \sim 0.35$ mag in the absolute magnitudes M_1 of the brightest members of rich clusters of galaxies measured by Sandage¹. In one view, advanced, among others, by Peebles^{2,3}, it is assumed that there is a universal cluster luminosity function, and that the value of σ_1 and of the variation of M_1 with cluster richness can be explained as the statistical result of drawing the brightest member from a set of clusters obeying this function. There is therefore nothing physically remarkable about the brightest member, and its luminosity is determined by the same mechanism as that which determines the luminosity of the fainter members. The opposing view, which has been advanced by Peach⁴, Sandage¹, and Tremaine & Richstone⁵, claims that the small dispersion is due to special conditions governing the formation or evolution of the brightest member, and is therefore entirely or in part independent of the luminosity function of the other cluster members. On this view one evokes either special conditions at the epoch of galaxy formation¹, or a mechanism, such as galactic accretion⁶, which governs the size and luminosity of the most massive galaxy in such a way as to give a small dispersion in the metric magnitude. The weakness of the statistical model, according to its opponents, is its inability to account for σ_1 without postulating a luminosity function steeper than those directly observed. The opposing view is open to the criticism that it is either *ad hoc* or that the mechanisms of galaxy evolution are too uncertain to lead to reliable conclusions. Here, we discuss the two interpretations in the light of new results and suggest that the case against the statistical model may not be as strong as sometimes suggested.

During a programme of photometry of clusters, we have measured the absolute isophotal magnitudes of the brightest members of the 12 rich clusters listed in Table 1. Details of the methods of observation and reduction are the same as those used in our previous work⁷⁻⁹; full lists of magnitudes will be published elsewhere, except for A1413, A1656 and A1930, which are already available. After the cluster name we give the redshift *z*, the cluster richness *R*, the radius r_{25} , defined as $(a/\pi)^{1/2}$ where *a* is the area contained within the standard

Table 1 Isophotal magnitudes for the 3 brightest members of 12 rich clusters of galaxies

Cluster	Redshift <i>z</i>	Richness class <i>R</i>	Isophotal radius (kpc) r_{25}	Absolute <i>V</i> magnitudes for the 3 brightest cluster members		
				M_1	M_2	M_3
Virgo	0.0038	0	43	-22.9	-22.4	-22.4
A426	0.0181	2	41	-24.1	-23.5	-23.3
A1377	0.0516	1	32	-22.9	-22.8	-22.2
A1413	0.1426	3	61	-24.6	-22.6	-22.6
A1553	0.1651	2	54	-24.1	-24.0	-23.7
A1656	0.0228	2	47	-23.9	-23.5	-23.3
A1930	0.1312	1	39	-23.4	-22.6	-22.2
A2065	0.0722	2	32	-22.8	-22.7	-22.5
A2147	0.0351	1	42	-23.2	-22.9	-22.6
A2151	0.0341	2	37	-23.0	-22.9	-22.8
A2199	0.0303	2	40	-23.9	-22.7	-22.6
A2670	0.0775	3	48	-23.9	-22.9	-22.9

isophote for the brightest member, and absolute V magnitudes M_1 , M_2 and M_3 for the first, second and third brightest cluster member measured to the 25 mag arc s⁻² isophote ($H_0 = 50$ km s⁻¹ Mpc⁻¹; $q_0 = +1$). Table 2 gives the dispersions in M_1 , M_2 and M_3 for the sample.

The absolute magnitudes M_1 of Sandage, on which the entire controversy has until now been based, have been derived by fitting photoelectric magnitudes measured through a series of circular apertures to a standard growth curve of magnitude against radius from the galaxy centre¹⁰. The small scatter of the observed points about the growth curve is claimed as evidence that the light distributions of the galaxies in his sample are similar to radii of ~ 40 kpc. The light contained within a standard metric radius of 43 kpc at the galaxy is inferred from the curve and gives, after absorption and K-corrections, the final metric magnitude. The second and third brightest, M_2 and M_3 , were not measured directly in this way, but their magnitudes were calculated on the assumption that the ratio of their luminosities to that of the brightest is given by the ratio of the apparent sizes of their images to that of the brightest; M_2 and M_3 are thus isophotal magnitudes. The dispersions σ_1 , σ_2 and σ_3 for Sandage and Hardy's¹¹ magnitudes for the 78 clusters in their sample and for the 12 clusters of Table 1 are shown in Table 2. It will be seen that the results for the present sample are not significantly different from those for the larger one. It will also be clear that $\sigma_1 = 0.57 \pm 0.18$ for the isophotal magnitudes is about one standard deviation larger than that for the metric magnitudes $\sigma_1 = 0.38 \pm 0.12$, whereas σ_2 and σ_3 are essentially the same. This difference cannot be attributed to measurement errors; we estimate our errors at about 0.2 mag which would imply a corrected dispersion $\sigma_1 = 0.53$ mag.

The reason for the greater spread in M_1 measured to a fixed isophote rather than to a fixed radius can be understood in the following way. The size of the brightest member to the 25 V mag arc s⁻² isophote r_{25} ranges from 32 kpc in A1377 to 61 kpc for the cD galaxy in A1413, with a mean of 42 kpc, fortuitously close to Sandage's standard metric radius. This radius is correlated with the isophotal magnitude in the sense that brighter galaxies tend to be larger, and fainter galaxies smaller, than the mean. As metric magnitudes are measured to a constant radius close to our mean, bright galaxies will possess brighter isophotal magnitudes than metric, whereas faint galaxies will possess fainter isophotal magnitudes, because of the difference in areas contributing to the integrated luminosity in the two methods of measurement. We find that for our sample

$$M_1 = -(7.3 \pm 1.5) \log r_{25} + \text{constant} \quad (1)$$

Sandage¹⁰ finds a dispersion in isophotal diameters, d , for 27 brightest members, after correction for measuring errors,

$$\sigma(\log d) = 0.075 \pm 0.02 \quad (2)$$

close to the dispersion of our sample 0.080 ± 0.025 . Our empirical relationship (1) when combined with (2) leads to a dispersion in the isophotal magnitudes of Sandage's sample of 0.54 mag which is close to our value. We have found that for reasonable functional forms of the radial luminosity distribution (1) is a likely consequence of correlations between the central surface brightness and the isophotal magnitude and between the scale-length of the distribution and magnitude, in the sense that brighter galaxies possess both higher central surface brightness and flatter luminosity profiles than fainter ones. There is some direct evidence for this in the work of Oemler¹².

Geller and Peebles³ have calculated in detail the predictions of the statistical model using a luminosity function based on Abell's observations of four rich clusters; this function also agrees well with one we have derived for the clusters of Table 1. Table 2 gives their values for σ_1 , σ_2 and σ_3 . Note that there is good agreement with our isophotal results but a disagreement with σ_1 for the metric magnitudes. They further predict a linear dependence of M_1 on richness class R with a slope of $dM_1/dR = -0.2$ mag per class. Sandage¹ has shown that for his larger

Table 2 Dispersions in the absolute V magnitudes of the 3 brightest members

Magnitudes		σ_1	σ_2	σ_3
Isophotal magnitudes from this work		0.57 ± 0.18 (12)	0.45 ± 0.14 (12)	0.45 ± 0.14 (12)
Metric M_1 and isophotal M_2 and M_3 from Sandage and Hardy ¹¹	All clusters	0.34 ± 0.04 (78)	0.51 ± 0.06 (74)	0.55 ± 0.06 (74)
	Clusters of Table 1	0.38 ± 0.12 (12)	0.49 ± 0.17 (10)	0.48 ± 0.17 (10)
Predictions of the statistical model (Geller and Peebles ³)		0.53	0.48	0.47

Errors are standard deviations. The number of galaxies in each sample is given in parentheses.

sample the actual variation must be much flatter. In our present sample of clusters, which is too small to give a good enough value for the slope to enable a direct comparison with the theory, $dM_1/dR = -0.21 \pm 0.25$ for the metric magnitudes and $dM_1/dR = -0.47 \pm 0.31$ for the isophotal. The latter is more than one standard deviation greater than zero and, if confirmed with a larger sample, would be difficult to explain other than on a statistical model.

The discussion of the statistical model has been carried further by Tremaine and Richstone⁵, who have derived two tests of the model which are more general than those of Geller and Peebles, in that they are independent both of the assumed form of the luminosity function and of its uniformity from cluster to cluster. Assuming solely an asymptotic behaviour of cluster luminosity functions certainly true of all observed functions, they prove that the two parameters

$$t_1 = \sqrt{\frac{N}{N-1}} \frac{\sigma_1}{\langle \Delta \rangle} \quad \text{and} \quad t_2 = \sqrt{\frac{N}{N-1}} \frac{\sigma_\Delta}{(0.677)^{1/2} \langle \Delta \rangle}$$

must be ≥ 1 for any large sample of clusters. Here, $\Delta = M_2 - M_1$, σ_Δ is its dispersion, $\langle \Delta \rangle$ its mean, and N the number of clusters. Their application of the test using the Sandage and Hardy magnitudes gave values of t_2 near unity, but t_1 was significantly less than unity for every sub-set of the data examined. Their conclusion was that no statistical model was likely to be valid. Using the isophotal magnitudes of Table 1, $t_1 = 1.0$ and $t_2 = 1.2$, a result consistent with the model. The change is mainly due to the increase of σ_1 . Although the present sample is small, the result again supports the view that the use of isophotal magnitudes may remove the discrepancy between observation and theory.

If the isophotal magnitude is a physically more significant parameter than metric magnitude, being more closely related to the mass of the galaxy, and if a larger sample of clusters confirms our result that the consistent use of isophotal magnitudes removes much of the conflict between the statistical model and the observations, it does not necessarily imply that there is nothing unique about the properties of the brightest member, only that any integrated photometric differences it may have from other cluster members do not exceed limits set by the model. It is possible that cD galaxies are structurally different from other giant ellipticals. There is also a suggestion that there is a steepening of the luminosity function in the denser parts of clusters with particularly bright brightest members, such as the Coma cluster⁹, similar to that predicted for galactic accretion; this is borne out in some of the luminosity functions of other clusters of Table 1. However, accretion on the brightest galaxy cannot have proceeded to the point where the galaxy has lost all those properties which are continuous with the properties of the fainter members.

If one accepts these conclusions, it is particularly difficult to maintain the view^{1,4} that the smallness of σ_1 is in some way due to special conditions not applicable to other members at the time of galaxy formation.

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Nucleation and growth studies by small angle neutron scattering and results for a glass ceramic

THE nucleation of phase separation in bulk solids remains one of the more elusive phenomena in both experimental and theoretical terms¹. Experimentally, the difficulties centre on the problem of gaining detailed structural information about the small composition fluctuations from which the precipitate develops. Theoretically, progress has been made in calculating stable microstructures in simple materials such as the rare gases² and amorphous metals³ as well as identifying transient clusters in liquids which are precursors to long range order and thus may act as nucleation embryos⁴. However, it remains a formidable problem to relate such calculations to the microstructure of a multicomponent solid or liquid. The most readily observed physical property of nuclei is their ability to facilitate growth of a new phase in appropriate conditions. Consequently, the study of the growth process immediately following nucleation is the most accessible though indirect method of inquiry into nucleation. It is, however, often difficult to follow the very early stages of growth either because it occurs too rapidly, or because the size or total mass of the precipitate falls outside of the conventional range of structural techniques. We report here the striking effects of nucleation as revealed by a neutron small angle scattering study (SANS) of a glass ceramic (2MgO, 2Al₂O₃, 5SiO₂ + 10% TiO₂ nucleating agent) during the early stages of growth of the precipitate. As a result of the continuous viscosity-temperature relationship, the rate of phase separation in glasses can be put on a favourable time-scale to follow the kinetics of growth simply by working at the appropriate temperature. Also as will be evident below, neutron small angle scattering provides important advantages in following the growth of precipitates in bulk. Three particular advantages have been exploited. (1) The range in scattering vector Q ($Q = (4\pi \sin \theta)/\lambda$) which it is possible to study includes the region around $Q = 0.005 \text{ \AA}^{-1}$ which generally falls between the scope of X-ray small angle scattering and light scattering measurements; (2) the low absorption of neutrons even at long wavelengths ($\sim 10 \text{ \AA}$) allows the easy investigation in transmission of a bulk glass which is heated directly in the neutron beam; and (3) the precipitating phase (an aluminium titanate solid solution) is rich in titanium atoms, and the negative scattering length of titanium ensures that there is good scattering contrast between the precipitate and the host silicate glass. All the experiments were carried out at the Institut Laue-Langevin on the D11 spectrometer⁵.

The general form of the scattering curves obtained is shown in Fig. 1; these curves were obtained on a glass nucleated by a pretreatment at 720 °C for 10 h. SANS is negligible immediately after nucleation and corresponds to the zero-time curve of

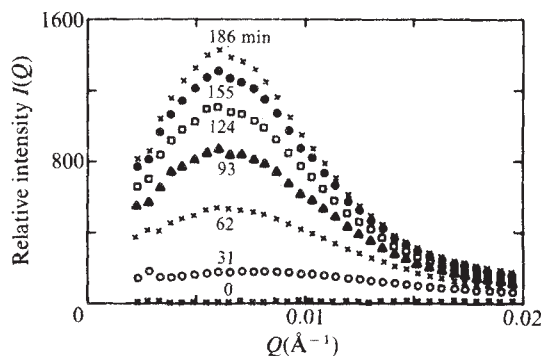


Fig. 1 Small angle neutron scattering curves from cordierite glass heated at 835 °C after nucleation pretreatment at 720 °C for 10 h.

Fig. 1. On heating *in situ* at 835 °C, SANS increases rapidly with the growth of the particles giving sufficient scattered intensity to allow the scattering curve to be followed every few minutes.

We emphasise some features of these curves which are characteristic of the particle nucleation condition before the onset of growth. The dominating feature of the curves is the pronounced maximum ($Q_M = 0.007 \text{ \AA}^{-1}$) with the intensity falling towards zero at low Q . Even at the earliest times for which the precipitate is very small, the maximum is apparent and the position remains stable during the growth period. The maximum is due to an interference effect between particles for which the spatial distribution is partly ordered. Similar effects are observed for the decomposition of alloys⁶. From the position of the maximum one obtains a rough estimate of the mean distance between the precipitates ($l \sim 2\pi/Q_M$) of 900 Å corresponding to a precipitate density of about 10^{15} cm^{-3} . We follow the development of the precipitates using Guinier plots on the high Q side of the maximum. The form of the scattering curve is, however, distorted by the interference term, so that the values obtained in this way are only approximate. Within the limit of validity $QR_g \leq 1.2$ the scattering function $\bar{S}Q$ is related to the radius of gyration R_g according to $\bar{S}Q = \exp(-Q^2/R_g^{2/3})$ and $R_g = (3/5)1/2R_s$, where R_s is radius of a spherical particle.

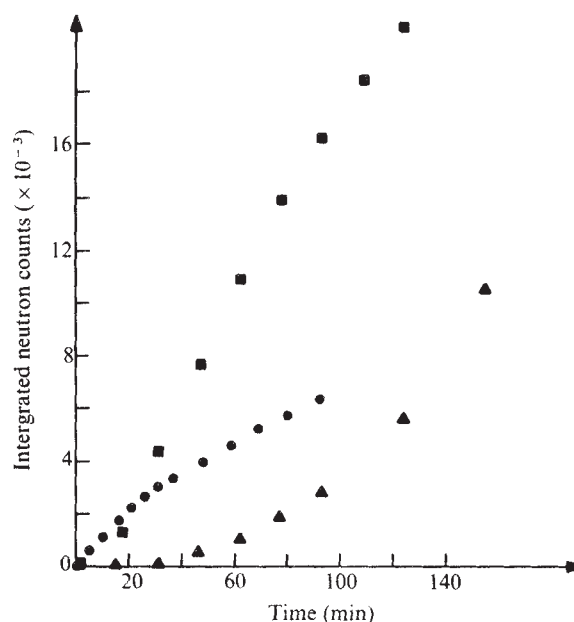


Fig. 2 Growth of integrated SANS intensity with time at 835 °C for different nucleation conditions. ■, 720 °C for 10 h; ●, 720 °C for 20 h; ▲, no nucleation treatment.

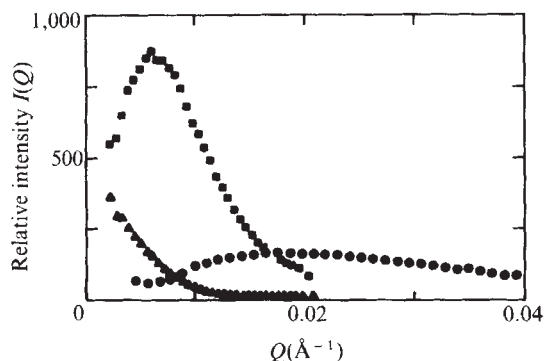


Fig. 3 SANS from cordierite glass heated for 90 min at 835 °C for different nucleation conditions. ■, 720 °C for 10 h; ●, 720 °C for 20 h; ▲, no nucleation treatment.

For the glass discussed above, R_c increases from 40 Å after 2 min to 210 Å after 180 min at 835 °C. There is an initial period of about 10 min (the induction period) where the SANS intensity increases very slowly, followed by a longer period (100 min) during which a rapid linear increase of intensity with time occurs before the rate slowly declines (Fig. 2). The intensity is a function of the volume of the precipitate and the contrast between the precipitate and the glassy matrix, which increases during growth as a result of the depletion of titanium ions from the glass. Indeed we observe that the intensity is proportional to the square of the volume of the precipitate which leads to the result that the scattering contrast is directly proportional to the degree of depletion of the glassy matrix.

When the nucleation pretreatment time is varied, different scattering behaviour occurs (Fig. 3). Allowing no nucleation time we observe an induction period of ~50 min (Fig. 2), when weak scattering occurs in the low Q region. Guinier plots here indicate the presence of particles of 600–800 Å. A second linear region at higher Q in the Guinier plot corresponding to a mean particle size of about 200 Å develops with time until it dominates the plots after 100 min, but there is no indication of an interference maximum in the scattering curve. The average particle size achieved is of the order of 500 Å, but the Guinier plots become nonlinear after long growth times, characteristic of a large distribution of particle sizes, or of irregular shaped particles.

If the nucleation time is increased to 20 h at 720 °C there is no induction period, but we observe a linear growth of intensity with time during the first 30 min before the rate declines slightly (Fig. 2). A broad but easily observed interference maximum is detectable after 5 min of growth, now centred at $Q = 0.018 \text{ Å}^{-1}$, corresponding to an inter-particle spacing of 350 Å or a particle density of $\sim 2 \times 10^{16} \text{ cm}^{-3}$. The particle sizes are now much smaller, being detectable at 20 Å and growing to only 55 Å after 100 min. The crystallised glass in this case is transparent, whereas non-nucleated and nucleated at 720° for 10 h are respectively white opaque and blue translucent after the crystallisation at 835 °C.

The significance of the results is that they provide information about the state of nucleation immediately before the growth. For the pretreatment of 20 h at 720 °C the immediate growth in SANS shows the existence of a large population of stable nuclei larger than the critical radius R_c . These are particles for which the Gibbs free energy will decrease with increasing size favouring immediate growth once the temperature is raised to give enough thermal energy for diffusion. The immediate presence of a maximum, however, indicates that the nuclei are present in an ordered manner, and gives a measure of the nucleation density. As the initial particle size is considerably smaller than the inter-particle distance, it is unrealistic to consider that the spacial order is due to development of extended excluded regions around the particles caused by depletion of growth material in the glassy matrix or by an Ostwald ripening

mechanism⁷. Such mechanisms would only generate an interference peak in the advanced stages of growth of the precipitate, so it is necessary to adopt an alternative hypothesis in which the homogeneous quenched glass undergoes structural changes during nucleation which lead to an enhanced probability that subsequent nucleation proceeds in an ordered or partly ordered manner.

The behaviour after a heat treatment time of 10 h is typical of many of the heat treatments we have performed, where an induction period is observed in the growth kinetics. It is reasonable (after Walton⁸) to consider that this period is composed of three parts

$$t = t_i + t_n + t_g$$

where t_i is the time to obtain a steady state in the cluster distribution, t_n is the time required for nucleation, and t_g is a part of the growth period where the particles are too small to be detected. Thus t_g does not influence the form of the growth curve, but simply makes the early part unobservable and so can be neglected. If we equate t_i with the time to develop concentration fluctuations, and t_n with the time to grow nuclei to the critical size then we can explain our results so far. During 10 h nucleation at 720 °C we develop metastable fluctuations or clusters in the glass, but fail to grow nuclei above R_c . On heating to 835 °C the microstructure is such that nuclei develop rapidly in only 10 min. The nucleation density is, however, much lower than after 20 h at 720 °C as many subcritical nuclei will redissolve at the higher temperature. This is shown by comparison of the size of the particles formed, although in no case have the particles reached the limiting radius. For the glass with no pretreatment we expect a small nucleation density arising from the imperfect quench from high temperatures. The results indeed show rapid growth of a few nuclei which disappear out of the lower end of our Q window, followed by the formation and growth of new nuclei at the end of a long induction period. In this case the induction period will include the time to establish a glass microstructure at 835 °C, followed by growth of the nuclei to the critical size. However, any nuclei present immediately begin to grow, generating a depleted region which limits the development of other nuclei. As we observe, the nucleation density is therefore smaller and disordered, and a wide particle size distribution in the crystals is seen.

These results show the scope of SANS in examining nucleation and growth in glasses. The technique we used, which as far as nuclei are concerned is analogous to a photographic development process, demonstrates that there are important structural changes taking place at temperatures below the glass transition temperature (T_g for this glass is 760 °C). In the cordierite glass system the possibilities for further examination of the time-temperature zone of nucleation are obvious. SANS in our view is the most promising way to explore the effects of the numerous catalysts (such as TiO_2 , ZrO_2 , and P_2O_5) which are employed in glass ceramic systems. Although in the present experiment we have been aided by the scattering contrast provided by titanium, SANS methods should be generally applicable to the study of nucleation and precipitation in glasses and probably other inorganic systems. Full details of the present work will be described elsewhere.

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The structure of α' -copper phthalocyanine and its susceptibility to radiation damage

CHANGES in electron diffraction patterns are a very sensitive monitor of the radiation damage experienced by a periodic specimen under observation in an electron microscope. In the study of α -copper phthalocyanine reported here, we have recorded the diffraction pattern of the a^*-c^* plane as irradiation proceeds, and from its behaviour we have been able to confirm the structure of the undamaged crystal and put realistic limits on the electron dose allowable if molecular images of a given resolution are to be obtained. We can also confirm the suggestion of Isaacson *et al.*¹ that the heavily damaged material forms a regular matrix, and that this matrix has approximately the same structure as that observed in previously published molecular images of this specimen^{2,3}.

The electron diffraction pattern produced by a crystalline specimen comprises a regular array of spots, the intensity of each being proportional to the square of the amplitude of the electron wave function at that point in the diffraction plane. If, somehow, the correct phase can then be ascribed to each amplitude, the wave function is completely determined and its form in any other plane may be calculated. In particular, the Fourier transform of the diffraction plane wave function may be calculated, and within the validity of the kinematic approximation, this is proportional to the electrostatic potential of a unit cell averaged over all the lattice sites. In the case of a perfect crystal this is identical to the potential of any individual cell, but as irradiation proceeds the system becomes a mixture of damaged and undamaged molecules. In these circumstances, although the Fourier transform of the wave function at the diffraction spot positions is still proportional to the average potential, this quantity need not be identical to the potential of any individual cell. Nevertheless, the concept of the average unit cell of such a mixture is a useful quantity. This is because the electron dose experienced by a radiation-sensitive specimen must be limited if there is to be a high probability that the molecules under observation are undamaged. When recording a high resolution image in these conditions very few electrons contribute to each picture element and so the intensities recorded will be substantially affected by statistical fluctuations. Thus in most studies of molecular images there must be some sort of periodic averaging to recover structure from these noisy intensities, and so the behaviour of the average unit cell with increasing irradiation dose is a valid guide to the molecular detail we can hope to obtain. Furthermore, electron diffraction patterns are less affected by lens aberrations than are electron images, and directly useful diffraction patterns may be recorded with very much smaller irradiation doses. We have therefore used diffraction patterns rather than images to study these crystals from the virtually undamaged state to that where no appreciable long range order remains.

Thin crystals of α' -copper phthalocyanine were produced by epitaxial deposition on a heated (300 °C) KCl substrate and were subsequently covered by a thin evaporated coating of

carbon. The carbon film-crystallite combination was then floated off on water. Electron diffraction patterns were obtained in a JEOL JEM 100C electron microscope and recorded on Kodirex crystallographic X-ray film. Subsequent densitometry was carried out on a Joyce Loebel flat bed densitometer, the intensities of each spot in the pattern yielding a quantity proportional to the square of the amplitude of the corresponding Fourier component of potential.

To determine the required diffraction plane wave function we must supply phases to each of these measured amplitudes. We proceeded by noting that the structure of platinum phthalocyanine has been determined and that the resemblance between its X-ray powder photographs and α' -copper phthalocyanine have led to the suggestion that their structures are similar⁴. However, recently published molecular images of α' -copper phthalocyanine have indicated that the molecules, whose shape is not in doubt (Fig. 1a), may be rotated with respect to those in platinum phthalocyanine^{2,3}.

To identify the correct orientation we examined these two possibilities to see which was consistent with our single crystal electron diffraction pattern data. Initially, we considered the structure isomorphous with platinum phthalocyanine and by assuming suitable theoretical potentials for each atom, calculated the theoretical diffraction plane wave function for this model. An experimental wave function was then constructed by combining the measured amplitudes with the calculated phases. If the phases are correct then the Fourier transform of this wave function should yield a structure very like that of the model. The resulting reconstruction (Fig. 1b) is indeed very similar to the model, individual atoms being resolved in many instances. When the orientation of the model is rotated through angles as small as 1° and the new phases combined with the measured amplitudes, agreement between model and reconstruction is less, and for rotations >5° parts of the molecule becomes unrecognisable in the reconstruction. Thus we are confident that α' -copper phthalocyanine has the same structure as platinum phthalocyanine and that the molecule is orientated with respect to the lattice as shown in Fig. 1b.

As irradiation proceeds individual molecules become damaged and deform. Although we do not expect them to do so in an identical way we would expect that because the molecules were originally centrosymmetric, the average unit cell of a mixture of damaged and undamaged molecules will also be centrosymmetric. As such, the phases attributable to each diffraction spot will still be 0 or π . Furthermore, as irradiation proceeds we would not expect to see a discontinuous change in the phase from 0 to π which would produce a change in the sign of the Fourier component of potential. Rather we would expect either a gradual diminution of the spot intensity to zero followed by a reappearance of the spot corresponding to a phase change, a gradual variation to a new value without falling to zero implying no phase change, or a gradual diminution to zero corresponding to loss of all long range order of that periodicity. In our study, although many intensities did vary appreciably, none passed through a zero before reappearing. Thus we believe that the original phases should be used in each of the Fourier reconstructions of the partially damaged crystals. Using this procedure we have recovered the average electrostatic potential after various electron irradiation doses (Fig. 1c, 1d).

As we can see, the behaviour is quite consistent with an increasing number of molecules becoming seriously disrupted, losing long range order of high resolution detail, but retaining some order at low resolution. The structure observed in (Fig. 1d) is strikingly similar to previously published^{2,3} molecular images and provides experimental confirmation that the technique is valid. In the diffraction pattern corresponding to this reconstruction, all but the first three orders of spots have completely faded and the remaining intensities are relatively stable. We conclude that this averaged unit cell corresponds to the radiation-resistant matrix of damaged molecules suggested by Isaacson¹, where although seriously disrupted, the material retains some periodic detail.

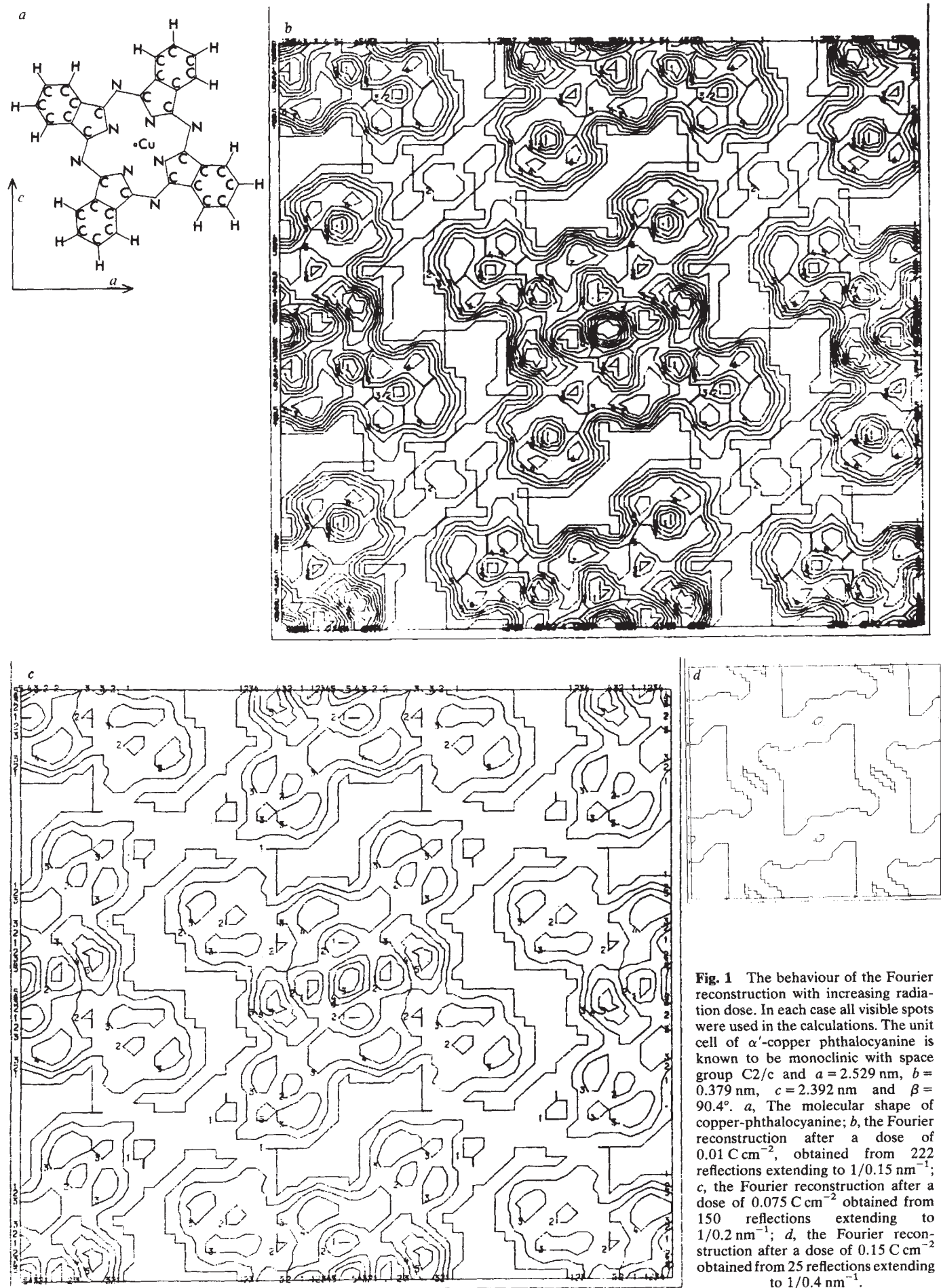


Fig. 1 The behaviour of the Fourier reconstruction with increasing radiation dose. In each case all visible spots were used in the calculations. The unit cell of α' -copper phthalocyanine is known to be monoclinic with space group C2/c and $a = 2.529$ nm, $b = 0.379$ nm, $c = 2.392$ nm and $\beta = 90.4^\circ$. *a*, The molecular shape of copper-phthalocyanine; *b*, the Fourier reconstruction after a dose of 0.01 C cm^{-2} , obtained from 222 reflections extending to $1/0.15 \text{ nm}^{-1}$; *c*, the Fourier reconstruction after a dose of 0.075 C cm^{-2} obtained from 150 reflections extending to $1/0.2 \text{ nm}^{-1}$; *d*, the Fourier reconstruction after a dose of 0.15 C cm^{-2} obtained from 25 reflections extending to $1/0.4 \text{ nm}^{-1}$.

From the behaviour of these reconstructions we suggest that for high resolution detail to be preserved the irradiation dose should be $<0.01 \text{ C cm}^{-2}$ and that the presence of periodic detail in images of this and similar materials recorded with greater values correspond increasingly to damaged material.

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Radiation pressure and 'aether drag' in a dispersive medium

ARNAUD¹ has pointed out that there should be no transverse 'aether drag' in an artificial dielectric consisting of parallel plane conducting elements, where the product of the phase and group velocities of electromagnetic waves should be equal to the square of the velocity of light *in vacuo*. This agreed with the formula derived by Player² and Rogers³, who for some important examples of aether drag showed that the Fresnel drag coefficient $(1 - 1/n^2)$, where n is the refractive index, should more precisely be replaced by $(1 - 1/n_\phi n_g)$, where n_ϕ and n_g are respectively the (phase, or normal) refractive index and the 'group' refractive index.

The same factor $(1 - 1/n_\phi n_g)$ seems to arise in the division of momentum into what may be described as 'electromagnetic' and 'mechanical' components when a packet of radiation enters a dispersive medium⁴. If the momentum of the packet *in vacuo* is p , the total momentum associated with the packet in the medium is $n_\phi p$, of which a portion p/n_g corresponds to the 'electromagnetic' momentum and $n_\phi p(1 - 1/n_\phi n_g)$ to mechanical momentum bodily in the medium. Thus, for any medium in which $n_\phi n_g = 1$ as in a wave guide or artificial dielectric, the mechanical component of momentum is zero, and the total momentum should be entirely electromagnetic.

The foregoing conclusion was derived by a thought-experiment involving a modified Einstein light-box to which were applied the relationship $E = mc^2$ and the consideration that actions inside a closed system cannot change the position of its centre of mass. Ginzburg and Ugarov⁵ have come to the same conclusion in a more detailed consideration of a particular case, where they conclude: "it is curious that for an isotropic (dispersive) plasma with $n^2 = 1 - (\omega_0^2/\omega^2)$, the equation G^M (the electromagnetic momentum) = G^A (the total momentum) holds." For it is also true that for such a plasma $n_\phi n_g = 1$.

The Ginzburg and Ugarov conclusion applies to a medium where the electrons are free, and not when they are bound as in a dispersive non-conducting solid. In that case some of the wave energy is carried as potential energy of electron displacement, and there is a consequent sharing of momentum. With free charges there is no corresponding potential energy of mechanical displacement from fixed centres and so it is at least plausible that there is no mechanical momentum drawn from the wave momentum. A common factor in the artificial dielectric and the plasma is the presence of free (inside the conducting elements) rather than bound electrons.

A corollary of the absence of mechanical momentum when $n_\phi n_g = 1$ seems to be that any medium satisfying this condition will also exert no 'aether drag'. A moving plasma in free space should therefore conform to this expectation, a conclusion also

reached by a more detailed argument for the particular case of a cold plasma by Ko and Chuang⁶.

A moving plasma can exert an aether drag, in apparent contradiction to the foregoing conclusion, when the plasma consists of moving carriers inside a semiconductor moving parallel to the direction of the light, as observed by Moss, Burrell and Hetherington⁷. I thank M. A. Player for resolving this contradiction—the solution lies in the fact that the plasma is now embedded in a medium of refractive index different from unity, with its faces in motion relative to the frame of reference in which the plasma is stationary. Such a situation, as pointed out by Einstein⁸ and Landsberg⁹, gives a modified Doppler shift, and when this is allowed for, a result consistent with Moss's observation is obtained.

An interesting variation of Moss's experiment would be to look for an aether drag effect when the free carriers in a semiconductor are moving transversely to the direction of the radiation.

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Plasma convection in force-free magnetic fields as a mechanism for chemical separation in cosmical plasmas

DURING the past two decades, progress in space research has forced us to abandon the earlier idea of a cosmical plasma as being a homogeneous medium. Filament formation and chemical separation are two important phenomena that are often observed in cosmical plasmas and illustrate the importance of inhomogeneity. An average composition from the Sun's atmosphere used to be taken as a standard when expressing cosmic abundancies in general. Numerous observations, however, of the chemical composition in the solar wind from *in situ* measurements and from lunar soil and meteorite samples, indicate a great variability in these ratios¹. The proton to α -particle flux ratio for solar flare energetic particle events varies widely between and within events². Recent observations show that this variability is connected with the flare process itself and the earlier history of the flare, rather than propagation effects between the flare site and the observer. Some local chemical differentiation mechanism in the preflare history of the specific active region is probably responsible for the composition of the emitted, energetic particles. The phenomena of filamentation and chemical separation may be coupled, as in the presence of a temperature gradient, the plasma convection associated with filamentary structures provides an effective means of selective transport. The general principles of this mechanism are described here. These principles may be important not only in solar flares but in many other cosmical plasmas where force-free fields and thermal gradients occur.

Typical filamentary structures, such as prominences and solar flares, can often be pictured as helical twisted magnetic flux tubes^{3–6}, where the magnetic field is approximately force free. The electric current flows almost in the direction of the magnetic field.

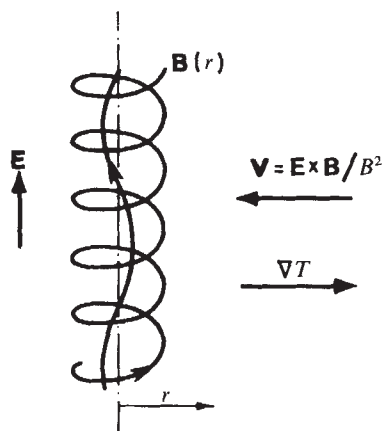


Fig. 1 General form of the magnetic field line pattern in a force-free axisymmetric filamentary structure. The ionised components of the plasma are convected inwards with a velocity V across a temperature gradient ∇T .

Consider for simplicity an axisymmetric case with a force-free helical magnetic field B , and a homogeneous electric field E applied parallel to the axis of the filament, to drive the current (Fig. 1). We assume that we have a transparent filament where the temperature decreases towards the axis of the filament due to a preferential cooling by radiation of the densest regions.

Under the influence of electric and magnetic fields the ionised component of the plasma will drift inwards from the surroundings towards the centre of the filament with a velocity $V = E \times B / B^2$, directed radially inwards. Some steady state models of filamentary structures, where this inward drift of plasma is balanced by an outward diffusion of neutrals, have been described elsewhere⁷.

This $E \times B / B^2$ convection is a very efficient process for collecting material to form the filament. This is true, even if the process is slowed by collisions, because as long as the particles are charged they are forced to drift inwards.

If the plasma is partially ionised—as in part of the solar atmosphere and many other cosmic plasmas—a temperature gradient will cause the radial transport to be different for elements with different ionisation potentials.

The most abundant elements of a cosmic plasma can be divided into groups of roughly equal ionisation potentials as follows: element, (approximate ionisation potential): He, (24 eV); H, O, N, (13 eV); C, S, (11 eV); Fe, Si, Mg, (8 eV).

Consider, for example, a case where the ambient plasma consists of hydrogen and helium in some initial proportion $p_0 = \{n(H) + n(H^+)\} / \{n(He) + n(He^+) + n(H_e^{2+})\}$ where $n(\)$ denotes the density of the element within the parentheses. In the configuration considered the ionised components of the plasma will drift inwards and experience a temperature decreasing with radius. When the charged particles have drifted so far that the local temperature is well below the ionisation potential of helium, the rate of recombination of ionised helium is considerable. The neutral helium produced is unaffected by the electric and magnetic forces that drive the ionised particles inwards. On the other hand, the hydrogen is still almost fully ionised, and is therefore still under the influence of the inward force. The protons will continue to drift inwards until they reach a radius where the temperature is well below the ionisation potential of hydrogen. Thus helium and hydrogen will be preferentially deposited in different regions (which may partly overlap). Compared with the composition of the initial ambient plasma, the above process will result in an enrichment of hydrogen in the inner parts ($p > p_0$) and of helium in the outer parts ($p < p_0$).

Although this discussion has considered a very simple configuration, the process works under very general circum-

stances. Any $E \times B / B^2$ convection in a multicomponent plasma will tend to separate elements of different ionisation potentials whenever spatial temperature gradients cause the different elements to leave (or join) the convection preferentially in different regions of space. Note also that the $E \times B / B^2$ convection can be a very rapid transport process compared with diffusion. (The latter will in general tend to counteract the separation. However, in thermally inhomogeneous, partly ionised plasmas, even diffusive processes can lead to non-homogeneous chemical distributions⁸⁻¹⁰.)

In the case of the flare process, the separation mechanism discussed above might be of importance for the great variability in composition observed. Because an equilibrium composition requires some time to get established in this way, an individual flare or the first flare in a series of flares will show a different composition of particles than that of a successive flare, which shows a composition characteristic of the partially re-established equilibrium. However, here the main purpose is to emphasise the existence of a separation mechanism intimately linked to force-free filamentary structure, and to describe the basic operative principles and potentially wide applicability.

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Persistence of rainfall anomalies in the central Pacific

THE behaviour of the atmosphere and ocean in the equatorial Pacific region, involving the 'Southern Oscillation' and 'El Niño' phenomena, has been the subject of many studies¹⁻⁵. Attention has been drawn to the notable persistence of anomalies for a year or more, the spatial correlation of sea surface temperature (SST), rainfall and pressure over large areas, and the strong correlation between atmospheric and oceanic features. Two types of mechanism may explain these phenomena and why they are so marked in that region: (1) thermal inertia, for example, some region of the ocean has a large capacity for heat storage, and the heat takes many months to build up or become depleted; (2) positive feedback, for example, if stronger winds in some region favour colder water, and colder water favours stronger

Table 1 Stations used in rainfall index

Station	Lat	Long	Period of data
Nauru	1° S	167° E	1941, 1945-71
Ocean I.	1° S	170° E	1941, 1947-71
Canton I.	3° S	172° W	1942-67, 1972-78
Fanning I.	4° N	159° W	1941-78
Christmas I.	2° N	157° W	1941-77
Tarawa	1° N	173° E	1948-78

Table 2 For each month: *a*, standard deviation of rainfall index *I*; *b*, regression coefficient of *I* on *I* for the previous month

Month	<i>a</i>	<i>b</i>
Jan	45	0.88
Feb	35	0.64
Mar	28	0.59
Apr	20	0.49
May	27	1.04
Jun	25	0.60
Jul	28	0.83
Aug	28	0.57
Sep	38	1.00
Oct	45	1.04
Nov	51	0.98
Dec	44	0.75

Data for 1941–78.

winds, then even if the thermal inertias of both features are such that their time constants are a month or less, anomalies can persist for many months⁶. New evidence is given here to support a feedback explanation.

Monthly rainfalls at the stations listed in Table 1 were obtained. A preliminary eigenvector analysis indicated that seasonal rainfalls at all these stations vary coherently. The monthly values were transformed by calculating cube-roots, to reduce the otherwise dominant influence of the wettest months on the analyses. Using the transformed data, each individual monthly value was then expressed as a percentage of the mean for that month over the datum period 1948–67. Finally, an index, *I*, was calculated for the period 1941–69 as the mean over stations 1–5, and for 1970–78 (when complete data for stations 1 and 2 were not available) as the mean over stations 3–6. Thus *I* is a series from which the annual variation has been removed, and in which no time smoothing has been performed.

Part of the series *I* is shown in Fig. 1*a*. Its persistence is remarkable, especially by comparison with almost any other meteorological series. Lag correlations for 1–6 months are, respectively, 0.79, 0.69, 0.56, 0.42, 0.31 and 0.20.

If the data are stratified by months, some interesting properties are evident. Table 2*a* shows that the variability of the index is least in April and then increases fairly steadily to 2.5 times its April value by November. Figure 2 shows that month-to-month persistence is quite strong throughout the year, and very strong during the period September–February. Also, there is notably strong persistence over several months from September to February, and weaker but still substantial persistence from June to the following March. Persistence is much weaker during

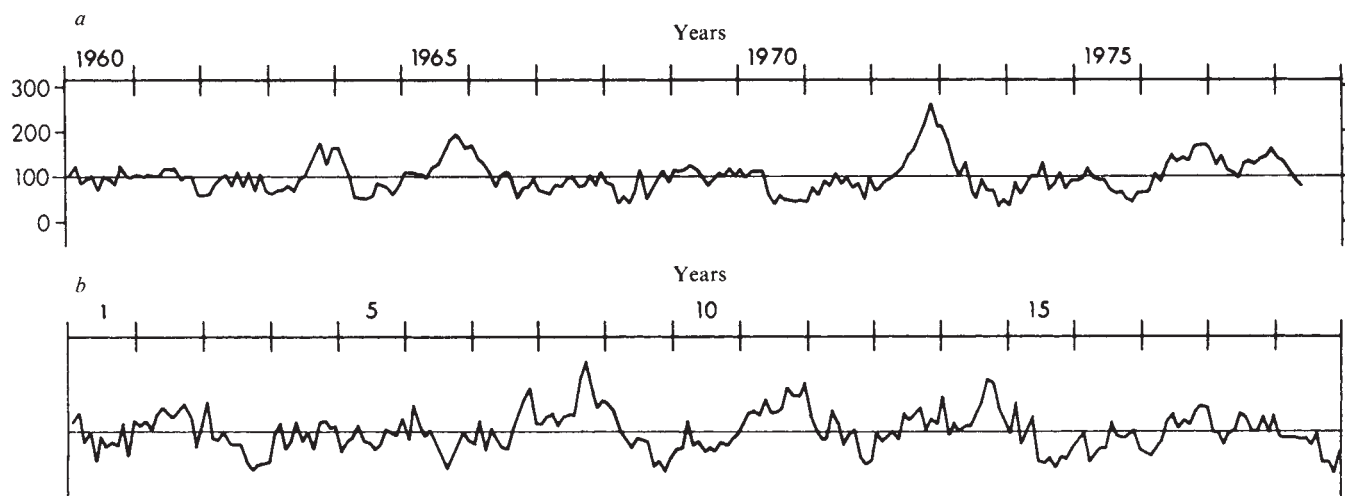
March to June. The breakdown occurs at the season of lowest variability; the greatest persistence occurs in the months of highest variability. These facts indicate that anomalies that develop initially around May to August tend not only to persist, but even to amplify. The amplifying property is emphasised by the regression coefficients of each month's index on the previous month (Table 2*b*), which are greater than unity in some months. Thus the regional rainfall has the following remarkable property: given the September anomaly, the best estimate of the October anomaly is an anomaly of the same sign and more extreme.

The pattern of negative lag correlation on the right side of Fig. 2 indicates a slight tendency for the anomaly to reverse in sign from one September–December to the next, and also a tendency for the sign in January–March to be reversed in the June–December of the following year.

In a typical year the rainfall anomaly in the region changes as follows. In April the anomaly is, in percentage terms, not very great. During May to August, either a dry or a wet regime tends to become established, with a steadily increasing tendency to persist. The regime during July and August is then likely to persist for many months, often until the following March. In particular, the regime in September is very likely to persist at least until January. If August is wet (dry), the next three months are not only likely to continue wet (dry), but also are as likely as not to be wetter (drier). From November to March the absolute value of the anomaly tends to decrease, and the regime shows an increasing tendency to break down. A new regime then tends to develop during April to August. The new regime is more likely than not to be the opposite of that prevailing in the previous year, and there is a hint that in some years, the new regime starts to show itself by January.

Table 3 As Table 2, but for 40 'years' of data generated by feedback model: *a*, standard deviation, *b*, regression coefficient

Month	<i>a</i>	<i>b</i>
(1)	31	0.42
(2)	28	0.10
(3)	24	0.16
(4)	21	0.31
(5)	29	0.67
(6)	26	0.41
(7)	34	0.70
(8)	43	0.91
(9)	56	1.11
(10)	51	0.79
(11)	44	0.68
(12)	36	0.61

**Fig. 1** *a*, Monthly values of rainfall index *I*, 1960–78. *b*, Monthly values of synthetic variable *A* for 19 'years'.

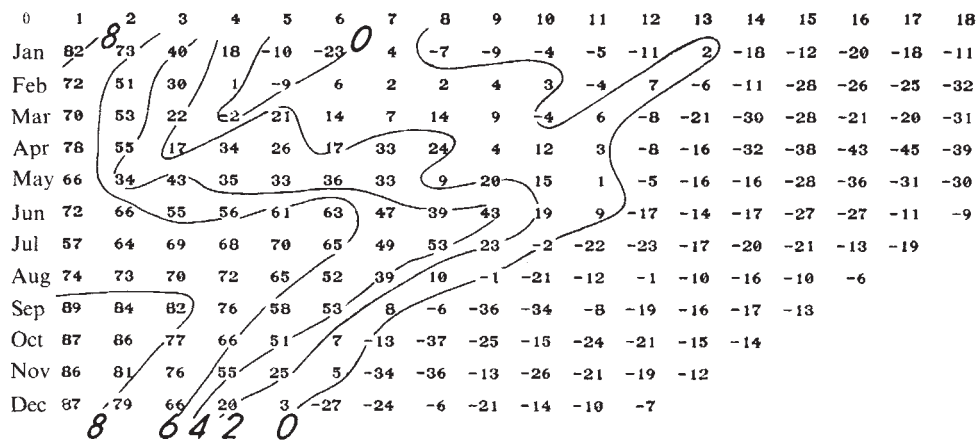


Fig. 2 Lag correlations of I in each month with I in the subsequent months. For example, January with February is 0.82; January with March 0.73; April with the following April -0.08. Data for 1941-78.

These relationships have obvious direct value for statistical prediction. It will be particularly valuable to monitor the development of the anomaly during May to September.

These properties of the rainfall series strongly indicate that some positive feedback mechanism, with the strength of the feedback varying through the year, is operating. To support this statement, I will examine the behaviour of a synthetic variable A involved in a feedback relationship with a second variable B , with external forcing F acting on A , according to the model:

$$\frac{dB}{dt} = -k_A(B - A) \quad \text{and} \quad \frac{dA}{dt} = -k_A(A - qB - F)$$

When F is constant, this system tends to equilibrium if q is <1 , and diverges if q is >1 . When F comprises a random series, the solutions exhibit fluctuations that are stable if q is <1 , and divergent if q is >1 , a property noted by Hasselman⁷. Here we consider the system

$$\Delta B = -0.02(B - A) \Delta t$$

$$\Delta A = -0.08(A - qB - F) \Delta t$$

in which the unit of time is taken to be 1/50 month, so the time constant of A (1/0.08) is a week, of B a month. F is the Markov series

$$F_k = 0.8F_{k-1} + E_k$$

where $[E_k]$ is a series of random numbers, so F has a spectrum similar to that of weather fluctuations. In this model A always influences B , while the influence of B on A (q) is given the values 0.4 in months 1-3, 0.8 (4-6), 1.3 (7-9) and 0.8 (10-12).

A sample of A is shown in Fig. 1b, and its statistical behaviour over 40 'yrs' is summarised in Table 3 and Fig. 3. The instability characteristic of months 7-9, when q is >1 , takes the form of strong persistence, increasing variability, and regression coefficients near or exceeding 1.0. This behaviour is similar to

that of the rainfall series I around October. Moreover, the general characters of the series I and A are similar. Closer similarity could be obtained by making appropriate changes to the constants in the model, but this has not been pursued. This comparison is sufficient to indicate that the behaviour of I can be explained as the result of a positive feedback relationship between two features with time constants of a month or less, in which the feedback is least around April and greatest around October.

For physical justifications, it must be shown that there are features whose mutual influences do have those properties. At present only a qualitative explanation can be offered. Rainfall in the equatorial central Pacific is least when the surface easterly winds there, that is, the Walker circulation, is strongest¹. Therefore I probably represents an index of the Walker circulation. A stronger Walker circulation favours more upwelling of cold water along the Equator due to Ekman divergence, and also stronger advection of colder water from the south-east Pacific; at the same time, colder SST in the equatorial-east Pacific implies a stronger surface temperature gradient along the Equator, which would favour a stronger Walker circulation⁸. This combination of mechanisms implies positive feedback, which could explain the persistence in I . However, there are other mechanisms of interaction between the Walker circulation and the SST; for example, strong Walker circulation is associated with decreased cloud cover in certain areas, which would favour warming of the ocean. Further, as the Walker circulation interacts with other atmospheric and surface features, additional feedback relationships may be involved. Any or all of these feedbacks might vary through the year; for example, stronger Walker circulation might favour stronger surface winds over the Indian Ocean in one season (favouring surface cooling) and weaker winds in another (favouring warming). All such interactions need to be identified quantitatively before we can claim a complete physical explanation of the statistical properties of I .

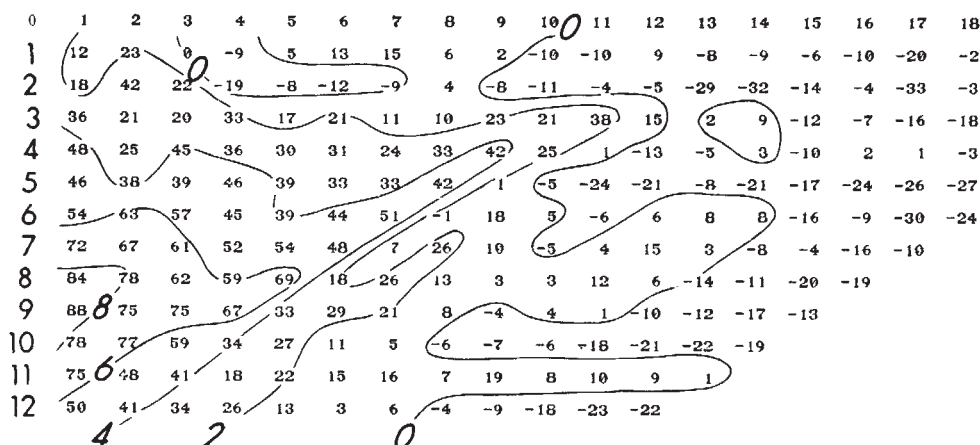


Fig. 3 Lag correlations of A (output of the feedback model) in each month with A in the subsequent months. Data for 40 'years'.

We have shown that rainfall anomalies in the equatorial central Pacific have remarkably high persistence during part of the year. The temporal patterns of lag autocorrelations and variability are readily explainable in terms of a positive feedback relationship in which the strength of the feedback is a function of season. Around October the feedback is so strong that the system displays the potential for 'runaway' behaviour. The feedback probably operates between the Walker circulation (on which the rainfall depends) and the SST, but studies of all the interactions between the Walker circulation and surface features will be necessary to confirm this quantitatively. Prospects are good for successful prediction; the index should be monitored particularly during May to September, because anomalies that develop then have a strong tendency to persist until the following February–March.

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Acoustic attenuation in Lake Tanganyika

THE Lake Tanganyika acoustic attenuation experiment of April 1970 (ref. 1) was the second of two lake experiments designed to help identify the cause of the low-frequency attenuation anomaly in seawater by providing comparative freshwater data. Although the Lake Tanganyika results showed no evidence of the anomaly, the attenuation was unaccountably large. Recent laboratory experiments have shown that the anomaly arises mainly from a chemical relaxation involving boric acid². We have been investigating pertinent chemical equilibria using the acoustic resonator technique³ and we have encountered another chemical relaxation-absorption in seawater⁴ that is evidently also responsible for the Lake Tanganyika results.

Table 1 Chemical relaxation data

	North Atlantic (concentrations in mg mol l ⁻¹)	Lake Tanganyika	Lake Superior
Mg	53	1.7	0.1
ΣCO ₂	2.5	7.2	0.5
Ca	10	0.3	0.3
SO ₄	27	0.03	0.03
B(OH) ₃	0.5	0?	0?
Temp. (°C)	4	23	4
pH	8.0	8.5	7.4

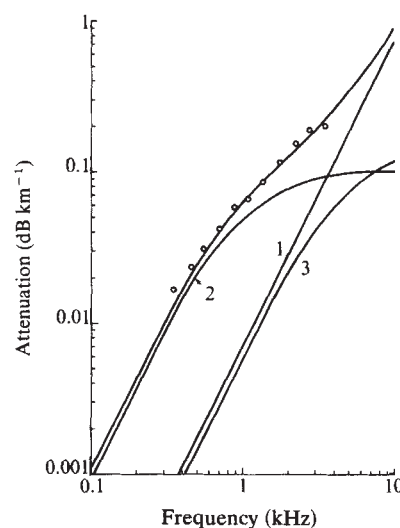


Fig. 1 Three relaxation components of sound absorption in the North Atlantic Ocean: 1, MgSO₄; 2, B(OH)₃; and 3, Mg/CO₂. The relaxation curves are of the form $\alpha_n = A_n f_n f^2 / (f^2 + f_n^2)$, where α_n is the component absorption coefficient (dB km⁻¹), A_n is a constant depending on the parameters, f is frequency, and f_n is the relaxation frequency. The top curve is total absorption and points are experimental field data.

Figure 1 shows the relative contributions of the three chemical relaxations in seawater, specifically in the North Atlantic Ocean deep sound channel. The newly discovered third relaxation involves magnesium and carbonic acid with calcium acting to reduce the component absorption by roughly a factor of two. Like that of the boric acid relaxation, the absorption varies as 10^{-pH} for pH < 9. (The nominal pH range in the world ocean is

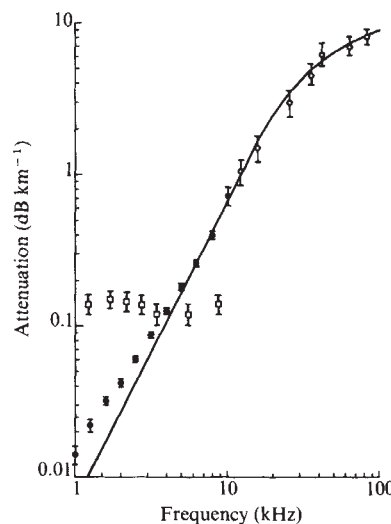


Fig. 2 Comparison of Lake Tanganyika (●) attenuation and synthetic lake water (○) results. The synthetic lake water constituents are MgCl₂, NaHCO₃ and CaCl₂ with appropriate values for Lake Tanganyika from Table 1. The pH is adjusted with NaOH. (Na and Cl concentrations are irrelevant.) The solid line is a relaxation curve-fit to the resonator data with relaxation frequency 40 kHz. The excess Tanganyika attenuation at low frequencies is believed to be caused by scattering. Lake Superior (□) also shows a frequency-independent scatter attenuation which is much greater because the water is shallower and the sound channel consequently weaker. Similarity to the B(OH)₃ absorption curve of Fig. 1 originally led us to the hypothesis that the seawater anomaly might also be present in freshwater which was, of course, contradicted by the Lake Tanganyika experiment.

7.7–8.3). The relaxation frequency varies from 4.5 to 40 kHz in the temperature range 0–30 °C.

Concentrations of the essential chemical components in the North Atlantic Ocean, Lake Tanganyika, and Lake Superior (the first lake experiment)⁵ are given in Table 1 together with temperatures and pH values on the respective sound channel axes. Figure 2 shows a comparison of the Lake Tanganyika attenuation and the laboratory resonator results with synthetic lake water. The solid line is a relaxation-absorption curve-fit to the resonator data. The small systematic excess seen in the Tanganyika data at low frequencies is believed to indicate an additional frequency-independent attenuation arising from diffusive scattering by random temperature inhomogeneities within the sound channel⁶. This mechanism is evidently solely responsible for the results of Lake Superior where the scatter loss is greater because of the weaker sound channel and the chemical absorption is negligible⁷.

We conclude that the acoustic attenuation in Lake Tanganyika is due primarily to a chemical relaxation-absorption involving magnesium and carbonic acid with a calcium moderator. Although neither the reaction nor even the chemical species involved are yet known, the results may be of wider interest than simply acoustical because of potential chemical and biological implications.

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Glaciation in Bolivia before 3.27 Myr

AN *in situ* layer of ignimbrite overlies till near the base of a thick sequence of glacial and fluvioglacial sediments at La Paz, Bolivia. Two radiogenic age determinations on the ignimbrite gave results of 3.27 ± 0.14 and 3.28 ± 0.13 Myr. We show here that this indicates that climatic cooling in the Southern Hemisphere was sufficiently severe to produce a mountain ice cap over part of the tropical Andes before 3.27 Myr and that these mountains had probably become tectonically elevated to about their present altitude by that time.

One of the most complete vertical sequences of late Tertiary to Recent strata in Bolivia is in the suburbs of La Paz where streams draining westwards and southwards from the Cordillera Real have cut gorges over 800 m deep into a variety of sediments (Fig. 1). Composed mainly of fluvial and fluvioglacial gravels, sands, silts and clay and till, with minor beds of lacustrine sediments and a layer of volcanic ash flow debris (ignimbrite), the deposits have been studied in most detail by Dobrovolsky¹. Table 1 summarises his interpretation of the sequence, which contains evidence of four major glacial episodes following the deposition of the La Paz Formation, a series of deposits of late Tertiary–early Quaternary age; the ignimbrite was erupted after the first glaciation.

A more recent revision of the area by Servant² subdivides the deposits rather differently but retains a similar sequence of four glaciations occurring after the La Paz Formation. One important departure from Dobrovolsky's interpretation is the identification

of two different beds of ignimbrite, one in the upper part of the La Paz Formation but before the first glaciation and a second resting on deposits of the first glaciation. He identifies these as the Chijini and Sopari ignimbrites respectively but does not describe them in detail; they appear only in his composite diagram of Plio–Quaternary stratigraphy (Fig. 2, on page 324 of ref. 2). Servant's analysis of the latter was based partly on his identification of four denudation surfaces in the north-west part of the Altiplano; he estimates that the oldest formed before the first glacial expansion from the Cordillera Real. A further proposal is that the oldest surface is the same age as an ignimbrite (Formación Pérez²) found in the western Altiplano 100–150 km west and south-west of La Paz. As this ignimbrite has an age of 2.5 Myr (Evernden *et al.*)³ the implication of Servant's work is that the first glaciation occurred after that date. These conclusions are based upon rather tenuous stratigraphical interpolation over large distances, however, and had Servant dated the two ignimbrites appearing in his stratigraphical diagram a more definitive statement could have been made concerning the age of his first glaciation.

I have recently encountered a small but new section in the northern suburbs of La Paz exposing a bed of ignimbrite overlying what appeared to be a layer of till at an altitude of ~3,900 m. The exposure is located in the gorge of a small left-bank tributary of the Rio Choqueyapu or Kaluyo (Fig. 1), the same valley in which Dobrovolsky observed his 'Chijini' ignimbrite overlying the oldest till.

The deposit beneath the ignimbrite is a very indurated layer of unsorted clasts up to 30 cm in size held in a compacted clay-like matrix; it averages 2 m in thickness. Out of three samples of 25

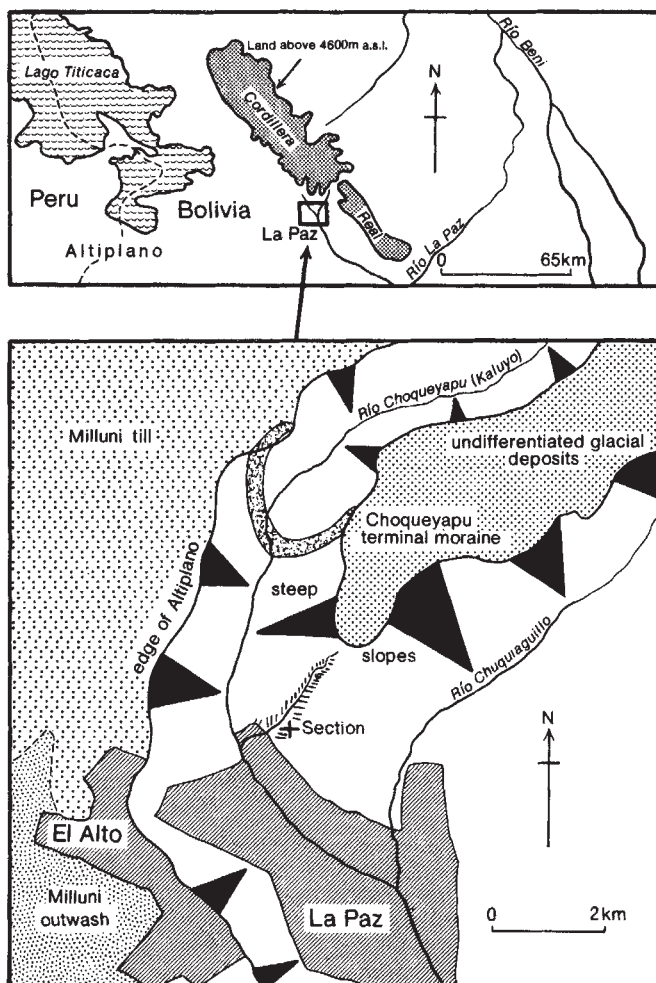


Fig. 1 Maps showing the location of the La Paz area in Bolivia and the site of the new section.

Table 1 Summarised description and interpretation of deposits in the La Paz districts of Bolivia*

	Period	Formation label	Description	Weathering	Maximum thickness (m)
Holocene	Post glacial	—	Landslide, mudflow, alluvium, etc.	—	—
	Glacial	Choqueyapu	Fluvioglacial outwash terminal moraines	Little weathered	200
	Interglacial	—	—	Erosion	—
	Glacial	Milluni	Fluvioglacial sediments till layer	Not deeply weathered	80
Pleistocene	Interglacial	Purupurani	Alluvial and lacustrine sediments including lignite >32,000 yr old	Intense erosion, plantation; upper 50 m (at least) oxidised	100
	Glacial	Calvario	Till layer	40 m depth oxidised, much eroded away	245
			Fluvioglacial sediments		85
	Interglacial	Chijini	Ignimbrite	Weak erosion intense erosion	16
	Glacial	Patapatani	Till layer	Most intense oxidation	15
Pliocene	Preglacial	LaPaz	Fluvial —upper —middle —lower sediments	Oxidisation	7
				Oxidisation	500

* After Dobrovlny.

stones, 70% were sub-angular in shape and 30% angular; several were well faceted and bore the polish and striations normally associated with glacial abrasion. More than 50% of the sampled stones were granitic, the rest being mainly hard sedimentary rocks; a few basic igneous stones were also present. Three samples of 25 stones with distinct long axes (ratio >2:1) were measured to see if there was a preferred alignment of the clasts. Fifty-six per cent of all stones were orientated within the arc of 120–140°, 92% lying between 120° and 170°; in other words a strongly preferred orientation that could be roughly classified as NW–SE or SE–NW. The former alignment would be entirely in keeping with the direction of ice flow at the base of an ice mass spreading outwards from the adjacent Cordillera Real (Fig. 1). The lower two-thirds of the deposit is a purple-grey colour whereas the upper third has been oxidised to a red-brown colour.

Because these characteristics and the stratigraphical position of this deposit are very similar to those of the lowest till identified by Dobrovlny ~5 km further up-valley, it is interpreted here as the same deposit. Dobrovlny considered this till and associated fluvioglacial sediments to have been much more profoundly oxidised than any of the subsequent overlying Pleistocene deposits and suggested that a considerable length of time elapsed before the eruption of the Chijini ignimbrite.

At the new section a bed of ignimbrite 2–3 m thick rests on the weathered upper surface of the indurated till. This volcanic deposit is a well known phenomenon of the La Paz district, outcropping as a distinctive single layer 2–15 m thick in the main gorges. Dobrovlny observed the ignimbrite above the oldest till in upper reaches of the valley but noted that it directly overlies the preglacial La Paz Formation elsewhere. A sample of the ignimbrite submitted for a radiogenic age determination gave the results shown in Table 2. Two radiogenic ^{40}Ar analyses were

performed on a pure biotite sample prepared from the ignimbrite. There is a close agreement between the two determinations, and McIntyre, who did the radiogenic analysis, considered that the measured age of 3.27–3.28 Myr is a good estimate of the volcanic event which produced the deposit.

The main implications of these age determinations concern the age of the La Paz Formation, the approximate timing of the tectonic elevation of the Cordillera Real, and the onset of full glacial conditions in these mountains.

Opinions on the age of the La Paz Formation (which is up to 500 m thick) have varied from Miocene to Quaternary, the uncertainty arising because of the lack of fossiliferous and other datable remains. Dobrovlny, however, after summarising all previous work, concluded that the scant fossiliferous evidence (the vertebrates *Scelidothereum* and *Parahipparion*) obtained from middle beds of the formation indicate a Lower Pleistocene age for these strata whereas the non-fossiliferous and coarser upper beds grade conformably into the first glacial outwash deposits. He tentatively assigned an Upper Pliocene age to sediments composing lower parts of the formation lying beneath a truncated deeply oxidised horizon which separates them from the middle beds. The fossiliferous evidence is so dubious, however, that it cannot be used as compelling evidence for determining the absolute age of the associated strata. If the radiogenic age of 3.27 Myr for the overlying ignimbrite is correct, then the entire sequence of the La Paz Formation is of Pliocene age.

As pebbles of granite from plutonic intrusions in the Cordillera Real are contained in lower beds of the La Paz Formation the mountains must have become tectonically elevated and eroded some time before 3.27 Myr. This agrees with Petersen's⁵ view that the latest phase of Andean uplift began in the Pliocene after the formation of the Puna plantation surface in Peru, Bolivia and Chile.

The spatial extent of till sheets (including the earliest) and piedmont moraines along western flanks of the Cordillera Real suggest that a mountain ice cap built up and discharged coalescent outlet glaciers 15 km beyond the foothills during periods of full glaciation. The earliest till layer, overlain by the dated ignimbrite, thus indicates that a substantial mass of glacier ice was present over these mountains more than 3.27 Myr ago. It is tempting to equate this glacial event with the first large-scale glaciation of the southern Andes dated at 3.5 Myr by J. H.

Table 2 K–Ar Analyses of biotite in ignimbrite, La Paz

K (wt %)	Mass (mg)	$^{40}\text{Ar}^*$ ($\times 10^{-7}$ SCC g $^{-1}$)	$^{40}\text{Ar}^*/^{40}\text{Ar}_T$	Age* (Myr)
7.11	104.9	9.037	0.222	3.27 ± .14
	91.7	9.070	0.243	3.28 ± .13

* Decay constants from Steiger and Jager⁴.

Mercer⁶. Such correlation may be reasonably valid because Dobrovolsky observed that the oldest till is intensely oxidised, suggesting a considerable time interval before the deposition of the overlying ignimbrite. Because till of the penultimate ice cap glaciation, exposed for possibly more than 100,000 years, is weathered to only a shallow depth, the intense alteration of the oldest till may well indicate a time interval of the order of 0.23 Myr. The first Bolivian glaciation would therefore most logically fit with the first Patagonian event.

Oxygen isotope and palaeomagnetic evidence from the Northern Hemisphere led Shackleton and Opdyke⁷ to suggest that the last lengthy period of stable sea level (global) and low ice volume (global) ended ~3.2 Myr ago. Mercer⁶, however, has since drawn attention to his evidence from Patagonia which shows that ice age cold prevailed in middle latitudes of South America 0.3 Myr earlier. This corresponds with recent work in the Barents Sea area of the Northern Hemisphere by Kvasov and Blazhchishin⁹ suggesting the existence of an ice sheet there 3.5 Myr ago. The deposits in Bolivia indicate that climatic conditions in the Southern Hemisphere had become sufficiently severe more than 3.27 Myr ago to allow the formation of an ice cap over a mountain range within the tropics. If the altitude of the Cordillera Real (present peaks between 5,000 and 7,000 m) has not been changed substantially by tectonic activity within the past 3.5 Myr, then the equilibrium line of the tropical ice cap was low enough to discharge outlet glaciers down to 3,900 m above present sea level. There is therefore an accumulation of evidence, deriving from polar regions^{9,10} and from middle and tropical latitudes of the Southern Hemisphere, which strongly suggests that the cold period of 3.5 Myr ago may have caused the first global large-scale glaciation of the Late Cainozoic ice age.

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A stratified water column environmental model for the type Kimmeridge Clay

GALLOIS has reported the widespread occurrence of coeval coccolith limestones and oil shales in the Kimmeridge Clay of southern England and Lincolnshire^{1,2}. From the association of these limestones with oil shale horizons he suggested that the organic content of the Kimmeridge Clay was largely derived from phytoplankton blooms whose decay produced the temporary anaerobic bottom conditions necessary to preserve organic matter. However, comparisons with similar Quaternary sediments in the Black Sea and Mediterranean has led us to report here that phytoplankton blooms were not the cause, but rather a symptom of widespread anaerobic bottom conditions,

and that preservational factors rather than productivity are the major control on the accumulation of black shales. We believe that the fine grained clastic-bituminous shale-oil shale-coccolith limestone lithologic association characteristic of the type Kimmeridge Clay can be attributed to the vertical movement of the O₂:H₂S interface in a temporarily stratified water column.

Figure 1 summarises the main features of the oil-shale and coccolith-rich portion of the type succession of the Kimmeridge Clay. The whole sequence was apparently deposited below wave base with only rare and indirect evidence of current activity, such as occasional low angle contacts exhibited by coccolithic streaks within sapropel rich horizons. The Kimmeridge Clay lithologies, with the exception of the early diagenetic dolomites, show a cyclic distribution pattern involving lithologies A-B-C-D (as on Fig. 1). Lithological changes through each cycle are accompanied by changes in faunal content. The clays (A) contain a fairly abundant low diversity macrofauna of small, thin shelled benthonic bivalves, plus a nektonic element including ammonites, fish and reptiles; the plankton includes dinocysts, coccoliths and rare acritarchs. In the bituminous shales (B) the macrofauna is similar to that of the clays, but it tends to occur only at discrete horizons.

In the oil shales and coccolithic limestones (C, D) benthonic fossils are rare or absent, and the non-carbonate organic content consists dominantly of amorphous sapropel with above average proportions of terrigenous palynomorphs^{3,4}. High concentrations of Cu, Ni, Mo, P, I and Br⁵ are associated with the organic matter. Preliminary scanning electron microscope studies have shown that the coccolithic laminae often contain well preserved coccospheres. Framboidal (probably early diagenetic) pyrite is ubiquitous in the coccolithic limestones, occurring in close association with organic membranes and palynomorphs.

The Quaternary sediments of the Black Sea and eastern Mediterranean basin also demonstrate an association of fine grained detrital, bituminous and coccolith rich lithologies (see Fig. 2 and legend for a summary of their principal lithologies). Radiometric dating of these sequences has enabled them to be matched with the climatic and physical oceanographic data for the Quaternary⁷⁻⁹, providing an insight into conditions that prevailed at the time of their deposition which we have applied to the Kimmeridgian.

The basal part of the Black Sea sequence (see Fig. 2: Unit Three) contains no marine macrofauna, and its dinocyst assemblage suggests that salinities of about 7‰ prevailed¹¹. Unit Two was deposited in marine conditions for it contains marine dinocysts, and marine sapropelic organic matter predominates over terrestrial humic material^{10,11}. The organic matter is associated with pyrite framboids and high concentrations of trace elements¹². The dark laminae within the coccolith oozes of Unit One have recently been interpreted as the products of seasonal mass mortality of the photosynthetic bacteria that live just below the O₂:H₂S interface in the Black Sea¹³. Cooling of the surface waters during the winter decreases the stability of the low salinity surface layer and enables turbulent mixing processes to reach down to the O₂:H₂S interface. The subsequent oxygenation of these lower waters causes mass mortality of the anaerobic bacteria. During the descent of the dead bacteria, the elemental sulphur which they contain probably acts as a nucleus for the formation of pyrite framboids within the water column¹³. Unit Three was deposited in a freshwater environment which was replaced by marine conditions between 10,000 and 7,000 yr ago when the post glacial eustatic rise caused the Mediterranean to spill over the Bosphorus Sill into the Black Sea. This produced a strong salinity-based stratification, and increased the productivity of the surface waters, the combined effects of which resulted in the stagnant bottom waters becoming rapidly anaerobic leading to the deposition of Unit Two. The O₂:H₂S interface rose rapidly from the sea floor until about 3,000 yr ago¹⁴, (see Fig. 2) when it reached a level where convective or turbulent mixing processes could provide its nutrients to the euphotic zone and hence stimulate the coccolith blooms which produced Unit One.

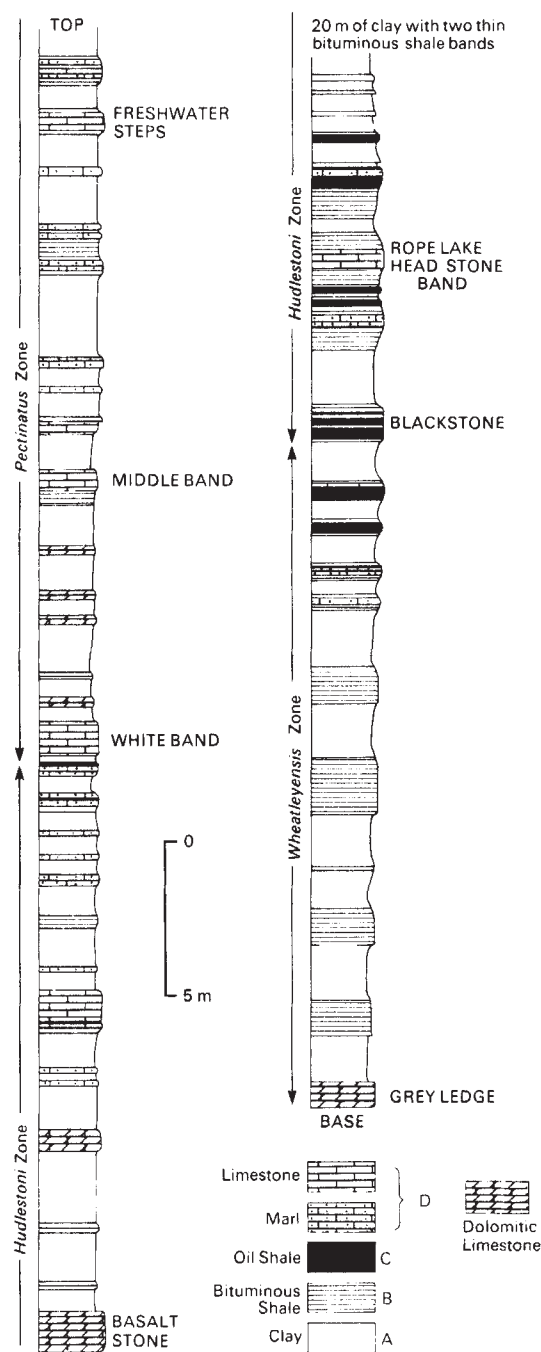


Fig. 1 A log of part of the type section of Kimmeridge Clay exposed in Kimmeridge Bay, Dorset. A, Clays—dominantly composed of illite, with minor silt sized material, up to 10% organic matter (predominantly amorphous sapropel), and $\leq 2\%$ pyrite. B, Bituminous shales—similar composition to the clays, but higher organic content ($\leq 30\%$). C, Oil shales—containing up to 70% organic matter. D, Coccolithic limestones—two varieties occur, one homogenised by bioturbation (for example, Rope Lake Head Stone Band), the other showing microlaminations of alternating dark coloured sapropel rich material and light coccolithic layers. Dolomites consist of a microcrystalline mosaic of ferroan dolomite, with amorphous kerogen either as dispersed fragments or discontinuous laminae (~ 0.25 mm thick). They formed over an extended period from early diagenesis onward by replacement of a pre-existing lithology⁶.

The best documented sapropelic sequences of the eastern Mediterranean are those of the Nile Cone^{8,16,17}, but similar deposits occur throughout the basin¹⁵. The sapropel sequences are between 30 and 50 cm thick, and are developed between basal terrigenous (turbiditic) sediments and overlying calcareous hemipelagic sediments; the principal lithological components are shown in Fig. 2. They occur below a present day water depth of 550 m, and are best developed in the distal parts of the Nile Cone. Olausson¹⁸ has developed a widely accepted depositional model for the eastern Mediterranean sapropels. The post-glacial amelioration of climate (which significantly altered the balance between evaporation and precipitation^{9,15}) coupled with the effects of freshwater overspill from the Black Sea, resulted in a strong halocline which reduced the efficiency of vertical mixing processes and led to the formation of anaerobic bottom water. The simultaneous transgression also caused large amounts of clastics to be trapped on the widened shelves⁸, so that there was minimal clastic dilution as well as enhanced preservation of organic matter.

The Quaternary examples imply that the Kimmeridgian sequence: clay-bituminous shale-oil shale-coccolith limestone may reflect the ascent of the $O_2:H_2S$ interface in a stratified water column. However, it is likely that the bottom water was just oxygen depleted for much of the time and did not always contain free H_2S . There are no indications of abnormal salinity in the type Kimmeridgian succession, and so dissolved oxygen was probably a more critical factor in determining the nature of faunal assemblages. The low diversity, thin shelled benthonic bivalve assemblage containing large numbers of small individuals, and the large planktonic component of the fauna are characteristic of low dissolved oxygen situations (~ 1.0 ml per litre O_2 , ref. 19). The small, thin-shelled, dominantly shallow infaunal bivalves are also characteristic of soft 'soupy' mud substrates, their morphology representing an adaptation to flotation within the sediment²⁰.

Figure 2 summarises the environment of deposition we envisage for each of the main Kimmeridge Clay sediment types. The clays were deposited under oxygenated bottom conditions below normal wave base, partly by hemipelagic settling and partly from low density suspensions. As 75% of the bivalves are articulated, any currents must have been slight, but the occurrence of infaunal species, apparently on bedding planes (as seen also in the Oxford Clay²¹) suggests that at least some exhumation by gentle currents occurred. The bituminous shales formed during periods of fluctuating aerobic/anaerobic conditions with a consequent preservation of greater amounts of organic matter, and the restriction of macro-benthos to discrete horizons. The oil shales were formed in predominantly anaerobic bottom conditions with only very rare current activity and minimal clastic sedimentation. They mark an intermediate phase in the upward progression of the $O_2:H_2S$ interface. The higher proportions of miospores in the oil shales^{3,4} (also seen in the Quaternary sapropels²²) and the sparsity of dinocysts probably indicate a low level of plankton productivity. The coccolith limestones mark the maximum development of anaerobic bottom water in the Kimmeridgian water column. At this time the top of the H_2S zone had migrated to a level where seasonal convective processes could provide its nutrients to the euphotic zone and there stimulate a coccolith bloom. In the upper part of the *Hudlestoni* Zone and the lower part of the *Pectinatus* Zone (see Fig. 1) there are many cycles in which the oil shale is missing but the coccolith limestone is present. Interpretation in terms of our model suggests a shallower depth of water than that lower in the sequence (where there are relatively thick oil shales), because in this case, once the bottom became anaerobic the $O_2:H_2S$ interface would take less time to rise to the thermocline and conditions suitable for oil shale formation would have been short lived. In the modern Black Sea, the most characteristic micro-laminated coccolith oozes occur in the halistatic parts of the basin where there is only a weak annual bloom (in spring, associated with seasonal convection²³) and minimal clastic

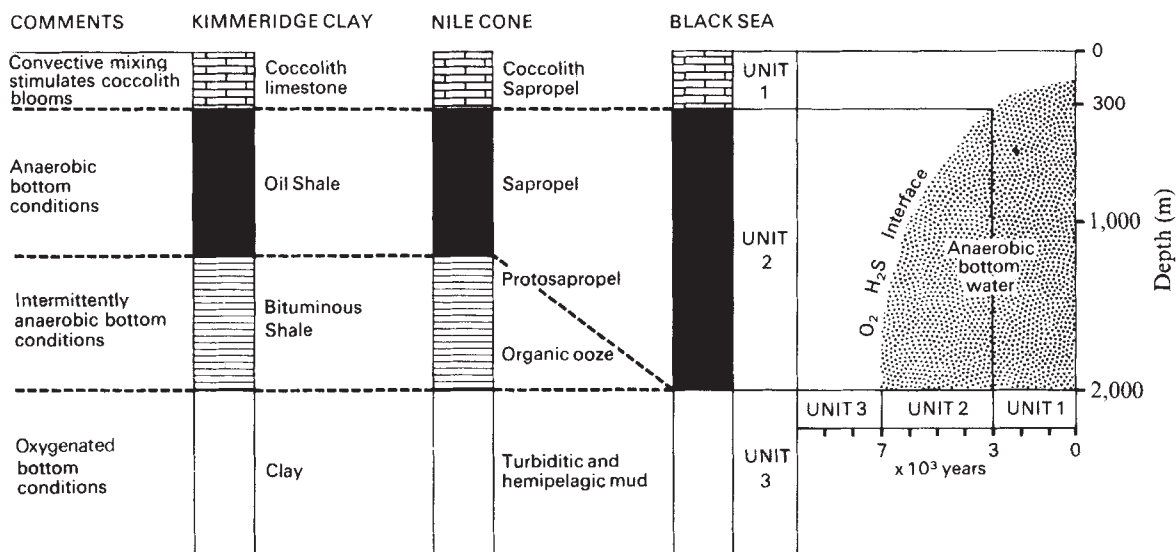


Fig. 2 Lithologies and environmental interpretations for the Kimmeridge Clay and Quarternary sequences discussed in the text. The sapropelic and cocolithic sequences of the Nile Cone and Kimmeridge Clay are interpreted in terms of the upward migration of the $O_2:H_2S$ interface documented in the Black Sea¹⁴, culminating in convective mixing of surface waters which stimulates cocolith blooms. Kimmeridge Clay lithologies are described in Fig. 1 and in the text. Nile Cone lithologies^{8,16,17}: organic ooze—with up to 15% sand grade biogenic material; protosapropel—bioturbated sediment transitional between organic ooze and sapropel; sapropel—black, organic rich ($\leq 15\%$) sandy mud, including planktonic forams, authigenic pyrite. Some sapropels contain interlaminated cocolithic and terrigenous muds. Benthonic organisms are absent. Black Sea lithologies⁷: Unit One, biogenic calcareous ooze, with light coloured cocolithic laminae alternating with dark organic rich laminae of detrital clays; Unit Two, micro-laminated dark brown gelatinous sapropelic clay with $\leq 25\%$ organic matter; Unit Three, partly homogenous and partly laminated light and dark banded lutites with interbedded turbiditic silts. Column sections are not to scale.

sedimentation. We believe that in this respect the Kimmeridgian is directly analogous. The preservation of the delicate laminations and coccospheres indicates that deposition was entirely by hemipelagic settling and that the bottom remained predominantly anaerobic during the deposition of the limestones. Dunn also believed, on the basis of pyrite distribution, that the cocolith limestones may have been deposited during the acme of anaerobic conditions²⁴.

Although the Kimmeridgian early diagenetic dolomites do not form part of the cyclothems, their development may be indirectly related to phases of watermass stratification. In the Mediterranean, precipitation of authigenic high Mg-calcites near the sediment surface shows an inverse relationship to the development of watermass stratification²⁵. This pattern is also reflected in the Kimmeridgian succession, for dolomites tend to be more common where the oil shales and cocolith limestones are poorly developed (that is, below the *Wheatleyensis* Zone and above the *Hudlestoni* Zone).

The Quarternary examples used as the basis for our Kimmeridgian depositional model represent deep water oceanic environments, whereas the Kimmeridge Clay was deposited in a shallower shelf regime: we submit that this apparent paradox is not a serious challenge to our model. For example, below the $O_2:H_2S$ interface in the Black Sea the environment is remarkably uniform and it is doubtful if the bottom conditions would be greatly different whether it was 2,000 m deep, or just a few hundred metres deep. Thus, although our model cannot add anything to earlier estimates of the palaeo-bathymetry of the type Kimmeridge Clay, it does suggest that the deepest water conditions occurred during the deposition of that part of the sequence where oil shales are most abundant (the top of the *Wheatleyensis* Zone and the bottom of the *Hudlestoni* Zone).

The development of widespread stagnant conditions in North-west Europe during the Kimmeridgian was influenced by both palaeogeography and a rise in sea level. The Lower Kimmeridgian probably marked the maximum extent of the Jurassic

shelf areas²⁶, and the increase in depth of the water column must have separated the mixed surface waters from the sea bottom, creating a stagnant, potentially anaerobic layer. At present, even relatively deep shelf seas are maintained in an oxygenated condition by wind driven currents and tidal mixing, and also by lateral circulation from the oceans. However, in comparison with its modern counterparts, the Kimmeridgian shelf sea was much wider, and the length of its oceanic (Tethyan) margin was small. Moreover, this margin was flanked by a carbonate platform in southern Europe which may have restricted the lateral shelfwards circulation of Tethyan water. The oxygenation of the internal shelf sea may, therefore, have depended largely on (seasonal?) convection and turbulent mixing in the surface waters, with the corresponding tendency for stagnation below wave base.

We can only speculate on what exactly controlled the cyclic ascent and decline of the $O_2:H_2S$ interface. Although in the Kimmeridgian the lithological response to stratification was almost identical to that in the Quarternary, it is clear that quite different mechanisms and controls were operative. The interpretation of the Quarternary examples revolves around the extreme climatic changes that occurred at the end of the last ice age, with glacial meltwaters and the land-locked nature of the basins playing a key role in the depositional model. Such an explanation is clearly incompatible with both Kimmeridgian palaeogeography and the more equable, sub-tropical climate of the period. Given this situation it seems probable that thermoclines, rather than haloclines, were the principal agents of water column stratification and stagnation in the Kimmeridgian. Quite small changes in local rates of subsidence, primary production, circulation pattern and climate may have combined to disrupt the fine balance between aerobic and anaerobic conditions to produce the short term cycles ($\geq 14,000$ yr)⁵ seen in the type Kimmeridge Clay.

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Reduction of radiation damage in an electron microscope with a superconducting lens system

OBSERVATION of biological specimens at high resolution by electron microscopy is limited by radiation damage. In general, structural details of non-periodical arrangements can only be identified for doses of radiation up to 10^{-3} C cm $^{-2}$, corresponding to 63 e nm $^{-2}$, which restricts the resolution to approximately 2 nm (refs 1, 2). Many methods have been applied in an attempt to reduce radiation damage, including high voltage, high vacuum and specimen pretreatment. The most successful, however, was the reduction of the radiation dose, used for certain specimens with periodical structure, where the information could be obtained by computer-aided image enhancement from underexposed films³. In special cases, although it was possible to obtain information at high resolution, the specimen was partly destroyed⁴. The advantages of low temperatures were also investigated, but due to the lack of an adequate instrument, no agreement between the results could be obtained. Furthermore, little attention was paid to the significance of the dose rate at low temperatures. We now have in operation an electron microscope with a superconducting lens system in which the specimen is cooled to liquid helium temperature⁵. The most important features of this instrument are the high resolution, which allows the transfer of space frequencies corresponding to the inverse of 0.15 nm, the mechanical and electromagnetic stability (specimen drift 0.01 nm min $^{-1}$) and the ultra high vacuum around the specimen. These features are necessary for testing the effect of radiation damage. We report here experiments which demonstrate that radiation damage is considerably reduced at low temperatures in a dose rate-dependent manner.

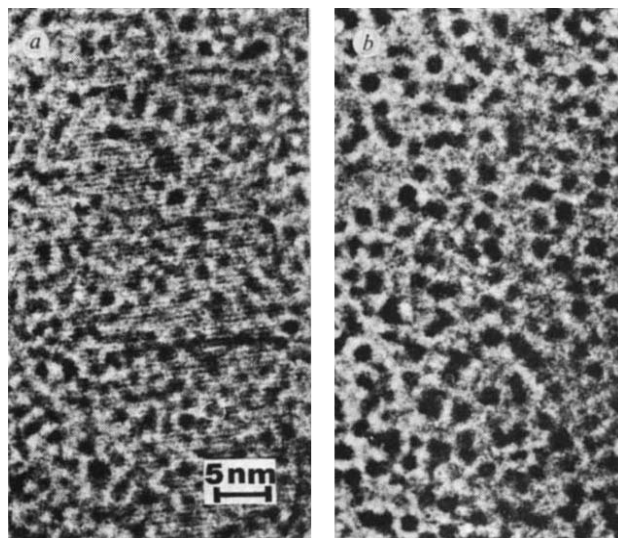


Fig. 1 Pt-stained murein or peptidoglycan, electron-optical magnification $\times 84,000$. For *a* and *b*, respectively, exposure time was 110 and 3 s, dose rate was 3×10^2 and 10^4 e nm $^{-2}$ s $^{-1}$, total dose during radiation was 3×10^5 and 1.5×10^6 e nm $^{-2}$, and estimated rise in temperature was < 1 K and ≥ 10 K. The phase structures in *a* and *b* are not completely identical due to a defocus difference of about 10 nm.

One of the specimens used was murein or peptidoglycan (the rigid layer of a bacterial cell wall), specifically stained with Pt. According to a model published by Formanek⁶, 0.45, 1 and 2 nm periodicities in the heavy-atom pattern of murein should be visible. The support was in most cases graphitic oxide, which shows very little phase contrast. In spite of extensive work, this structure has not been seen previously in the electron microscope.

Figure 1*a*, taken with a dose rate of 3×10^2 e nm $^{-2}$ s $^{-1}$ (0.005 A cm $^{-2}$) clearly demonstrates, even after a total irradiation dose of 3×10^5 e nm $^{-2}$ (5 C cm $^{-2}$), the presence of 0.45-nm periodicities, which disappear as soon as the dose rate is increased to 10^4 e nm $^{-2}$ s $^{-1}$ (0.2 A cm $^{-2}$) (Fig. 1*b*). Light optical diffractograms of Fig. 1*a* show reflections corresponding to periodicities of both 0.45 nm and 1.0 nm. Such reflections were also detected on carrier-free murein.

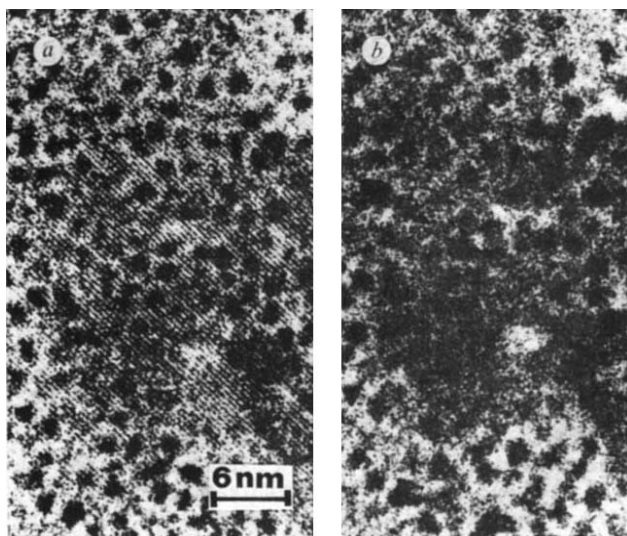


Fig. 2 Pt-stained polyhydroxybutyric acid, electron-optical magnification $\times 84,000$. For *a* and *b*, respectively, exposure time was 120 and 16 s, dose rate was 3×10^2 and 5×10^3 e nm $^{-2}$ s $^{-1}$, total radiation dose was 1.4×10^6 and 2.5×10^8 e nm $^{-2}$, and the estimated rise in temperature was < 1 K and ≥ 10 K.

We also investigated a Pt-stained by-product of the murein preparation. Periodicities were clearly visible as long as the dose rate was kept below a certain critical value (Fig. 2a) and faded away when the dose rate was increased (Fig. 2b). We have assumed that the material is poly- β -hydroxybutyric acid, as this is the only possible crystalline organic contamination originating from the bacterial cell. In addition, a periodicity of 0.36 nm agrees with one of the main lattice distances of poly- β -hydroxybutyrate⁷.

The observations that the conservation of the fine structure of the biological material strongly depends on the radiation dose rate we consider to be due to a temperature effect. The influence of the dose rate on the specimen temperature can be estimated⁸. For the compounds under discussion the chemical bonds are destroyed during the first exposure; however, the diffusion of the molecular fragments and especially the heavy atoms is prevented if the specimen is kept at a sufficiently low temperature. At the critical dose rate, the temperature is increased above the threshold value that permits diffusion. Comparison with the results obtained at room temperature shows that when specimens are observed close to liquid helium temperatures, damage to their biological fine structure is dramatically reduced.

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Recent archaeological findings at the Sterkfontein site

UNTIL the present decade it was thought that the Early Stone Age of Africa consisted of two industrial complexes, the Oldowan and the Acheulian, the latter succeeding the former after a transitional phase. Well excavated and well dated Oldowan sites are known only from Eastern Africa, although Oldowan sites are reported from North and South Africa, and all known examples are older than 1.5 Myr (refs 1–4). The earliest manifestations of the Acheulian appear approximately 1.5 Myr BP (refs 5, 6) and continue until the Last Interglacial. Leakey has challenged the idea that the Acheulian represents a continuous development of the Oldowan in East Africa^{1,7,8}. At Olduvai in Bed II she recognises two industrial complexes, which are said to represent two distinct cultural traditions, possibly produced by two different hominid taxa. These industries are the Acheulian and the Developed Oldowan. The criteria for defining the Developed Oldowan B have recently been revised and Leakey⁷ now states that the morphology and

technical features of manufacture of bifaces are the principal distinguishing characters between the two industries. The main technological distinction is that the Acheulian biface makers knew how to detach large flakes (>10 cm) while the Developed Oldowan hominids did not. As a result of this revised definition some of the assemblages at Olduvai originally defined as Developed Oldowan B have been reclassified as Acheulian by Leakey⁷. The problem of the Acheulian and Developed Oldowan can be stated as two alternative hypotheses. (1) Two cultural traditions began in East Africa approximately 1.5 Myr BP, each associated with a distinct social population over an immense span of time; possibly they were of different hominid taxa. Or (2), there was only one cultural tradition (industry), the Acheulian Industrial Complex, beginning about 1.5 Myr BP and marked by highly variable bifacial tools and relative frequency of artefact types; only one hominid taxon during any given period was involved. I have now made a comparative study of the Sterkfontein and Olduvai Gorge Bed II assemblages, which suggests that the Developed Oldowan B industry falls within the Acheulian Industrial Complex and therefore serious consideration should be given to dropping the former term.

To test the hypotheses four assemblages were selected for comparative analysis from Bed II at Olduvai Gorge, two defined Early Acheulian, EF-HR and TK Lower Floor (TKLF), and two defined Developed Oldowan B, TK Upper Floor (TKUF) and FC West Floor (FCWF). These assemblages were chosen because each is made up of material excavated from a single occupation floor, and therefore each represents a chronologically homogenous set of artefacts presumably produced by one social group of hominids. The other Bed II assemblages do not satisfy this criterion. Table 1 presents the shaped tool type percentages of the four assemblages, demonstrating clearly that the original criteria of the Developed Oldowan are no longer valid. TKLF, an Acheulian assemblage, has a high percentage of spheroids/subspheroids, the most varied light-duty tool component, and a low percentage of bifaces. This assemblage is considered Acheulian by Leakey⁷ because of the technique of manufacture of the bifaces. The conclusions of the comparative study will be restricted to bifaces, the taxonomically critical type. Bifaces from the Sterkfontein site are shown in Fig. 1.

Nine attributes out of a larger set were selected to describe the morphological and technical features of the bifaces. The results are presented in detail elsewhere^{9,10}, including analysis of other artefact types. The application of non-parametric statistical tests (χ^2 and Mann-Whitney *U* test), measures of the coefficient of



Fig. 1 Sterkfontein bifaces. a, Handaxe from cavity infilling of Robinson excavation; b, handaxe from overburden; c, cleaver from overburden; d, handaxe from overburden; e, handaxe from overburden; f, handaxe from Dump 1. Illustrations by Zoë Martin.

variation, and computer assisted multivariate programs (average-linkage cluster analysis and discriminant analysis) were made of the five assemblages using the attribute values of the bifaces and resulted in the following conclusions. (1) The bifaces of the defined Acheulian assemblages were significantly different from those defined as Developed Oldowan B; (2) the Acheulian bifaces were longer, thicker, and had longer working edges; (3) the Acheulian bifaces were made significantly more often on large flakes (>10 cm); (4) the differences observed between the bifaces of the two industries were due to the difference in frequency of use of large flakes as the primary form for biface manufacture; and (5) the Acheulian bifaces display an equal degree of standardisation within assemblages as the Developed Oldowan B bifaces.

Table 1 Shaped tool frequencies of four Olduvai Gorge Bed II assemblages

	Site							
	EF-HR		TKLF		TKUF		FCWF	
	No.	%	No.	%	No.	%	No.	%
Choppers	14	17.7	6	5.1	29	10.2	49	25.9
Proto-bifaces	0	0	0	0	0	0	0	0
Bifaces	37	46.8	9	8.0	18	6.3	4	2.1
Polyhedrons	5	6.3	1	0.9	3	1.0	4	2.1
Discoids	8	10.1	0	0	9	3.2	4	2.1
Heavy-duty scrapers	3	3.8	6	5.1	13	4.6	11	5.8
Spheroids/subspheroids	9	11.4	28	23.9	76	26.7	48	25.4
Modified battered cobbles, nodules and blocks	0	0	17	14.7	35	12.3	54	28.6
Light-duty scrapers	3	3.8	24	20.5	78	27.4	9	4.8
Burins	0	0	12	10.3	6	2.1	1	0.5
Awls	0	0	8	6.8	15	5.3	2	1.1
Outils écaillés	0	0	2	1.7	0	0	0	0
Laterally trimmed flakes	0	0	4	3.4	3	1.0	0	0
Sundry tools	0	0	0	0	0	0	3	1.6
Totals:	79	99.9	117	100.4	285	100.1	189	100.0

χ^2 Tests of the unmodified whole flakes demonstrated that large flakes in general were present significantly more often in the Acheulian assemblages. Because large flakes were present in the Developed Oldowan B assemblages, Leakey's assertion that culture tradition explained the inability of the Developed Oldowan B peoples to produce large flakes could not be immediately accepted. The alternative explanation of differential use of raw material was investigated.

Use of χ^2 tests showed that large flakes were produced significantly less often from quartz than from lava. Bifaces made from flakes and unmodified whole flakes were of quartz significantly more often in the Developed Oldowan B than in the Acheulian assemblages. These findings suggested that the basic difference between the Acheulian and Developed Oldowan B bifaces was the differential use of quartz and lava as raw material. In the absence of any other archaeological evidence, I suggest that the latter hypothesis be accepted—that is, that the Acheulian Industrial Complex, beginning about 1.5 Myr BP, was marked by highly variable bifaces and per cent frequency of artefact types. The term Developed Oldowan B should be dropped unless archaeological evidence can be produced to establish its independence from the Acheulian.

Using the BMDP 2M average-linkage cluster program on the CDC 6400 computer at the University of California, Berkeley, I found that the Sterkfontein bifaces clustered exclusively with the Acheulian bifaces of EF-HR, but that when assigned as ungrouped cases to the Olduvai assemblages in the discriminant analysis (SPSS program with Mahalanobis D^2 as the distance measure) they were more often grouped within the Developed Oldowan B of TKUF. The Sterkfontein bifaces were also found to be more highly standardised as assessed by the coefficients of variation of biface attributes and the discriminant analysis

results^{9,10}. A comparison of univariate frequency distributions of attributes of several different artefact types showed Sterkfontein to display no consistent patterns of similarity or dissimilarity with any particular assemblage of the Olduvai industries, but rather it presented an overall pattern of distinctive variability. I conclude that the Sterkfontein assemblage was Early Acheulian and that it displays characteristics that fall well within the range of those of the Acheulian complex of East Africa.

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Jackal helpers and pup survival

BLACKBACKED jackals (*Canis mesomelas*) are one of the few mammalian species to have long-term pair bonds and to exhibit a tendency for some offspring to help in the provisioning and guarding of subsequent litters. Bonded pairs of adults were studied for 28 months over 3½ years at Lake Ndutu, Serengeti Plain, Tanzania. In the 15 litters observed, families with helpers had significantly higher offspring survivorship.

The blackbacked jackal is a small omnivore that typically occurs in brushland habitat^{1–4}. Bonded pairs are territorial and both the female and the male mark and defend boundaries. Bonded pairs groom each other, hunt cooperatively³, and share food. Parental care of pups is approximately equal. Both of them feed and guard the pups and the male regurgitates to his mate during the period of lactation. In all observed boundary fights the aggression occurred between same sex individuals. The territories form a mosaic with core territories in the centre of the *Acacia* sp. and *Balanites* sp. woodland surrounding Lake Ndutu, and peripheral territories adjacent to the short grasslands. Three of four pairs of blackbacked jackals identified in July 1974 still occupied approximately the same territories in February 1978. In August 1976 an additional five pairs were identified.

Eleven out of 15 litters observed had non-parent adult helpers. In 1976–77, five families were observed during two consecutive litters. In 1977, four of these families had helpers and the 'helpers' were pups from the previous year's litter. Thus with monogamous parents the helpers were full siblings to the litter of pups that they were caring for. Given this genealogy, the degrees of relatedness are as follows: parents to offspring, $r=1/2$; helpers to full sibs, $r=1/2$; parents to grandpups, $r=1/4-1/2$ (dependent on degree of inbreeding); helpers to offspring, $r=1/2$.

Presence of helpers may be partially dependent on the age of the parents. A pair with its first litter would not have any helpers. However, the survival of pups does not ensure the presence of helpers the next year. In 1976–77, four families had 12 surviving offspring. Of these, four stayed as helpers (1 per family). In the fifth family, four pups survived and none remained as helpers.

Helpers contribute food directly to the pups, and also regurgitate to the mother during the period of lactation. The percentage of observed regurgitations by helpers to pups varied from 18 to 32 within families. Helpers also contributed by guarding the pups while the parents were absent. Spotted hyenas do attempt

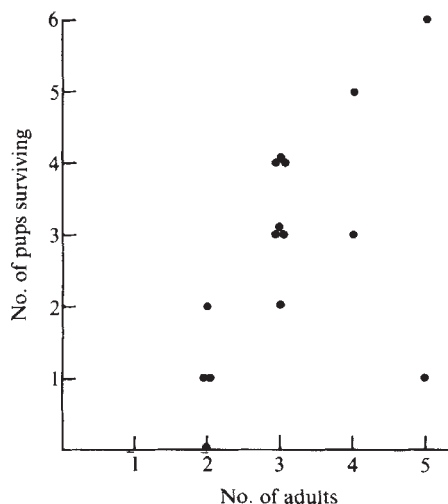


Fig. 1 Number of adults per family and pup survivorship. $n = 15$; Spearman rank correlation coefficient, $R_s = 0.967$; $P < 0.01$.

to prey on young pups, but can be successfully driven away by one adult jackal. Families with more adults (that is, those with helpers) had an attendant adult at the den a higher proportion of the time, thus providing greater protection for the pups. Helpers also play, groom, and help teach the pups to hunt. Quantitative details of helper contributions will be published elsewhere.

Pups first emerge from the den at approximately 3 weeks of age and are weaned at 8–9 weeks. By 14 weeks they are well coordinated, no longer use a den, and are starting to forage with adults. All observed mortality has occurred within this initial 14-week period. This is the time within which the pups are most vulnerable to food deprivation and predation, and the time period in which major contributions are made by helpers and parents.

The presence of helpers correlates positively with pup survivorship and was significant to the 0.01 level (Fig. 1). On average, each helper added 1.5 surviving pups to the litter. One aberrant group (litter number 15) had three helpers initially and only one surviving pup. This is the only family in which a helper has been observed to remain for more than one year, and the parents were old. At 14 weeks of age, only one pup remained, and was cared for almost exclusively by the second year female helper. The other two helpers had disappeared. Thus this family had old parents, and for part of the critical period for pups, only one helper.

The presence of helpers seems to be independent of food density. In the study area, jackal territories in the core and periphery of the woodland vegetation differ in the density of an important prey species, the grass rat (*Arvicanthus niloticus*). In a core territory (VIII), trap-recapture data on *Arvicanthus* density varied from 7,792 (November 1976) to 32,083 rats km^{-2} (July 1977). In a peripheral territory (VII), *Arvicanthus* varied from 3,125 (November 1976) to 13,125 (July 1977) (R. Senzota, personal communication). The ratio of rat density between core and peripheral territories was 2.5:1. However, this difference in food availability did not seem to affect the presence of helpers and pup survivorship. In territory VIII, a core territory with high prey density, the resident pair with one helper had four pups survive from the 1976 litter. None of these pups stayed as helpers. In June 1977, the same pair had a litter of five pups, and none of them survived. By contrast, in 1976, in peripheral territory VII, the resident pair with no helper had a litter of five pups and one survived. This pup remained with his parents. In July 1977, the same pair had a litter of six pups and one helper. Three pups survived. Even in the peripheral area with the lower *Arvicanthus* density, there is still a large biomass available at whelping time ($13,125 \times 60 \text{ g} = \text{approximately } 800 \text{ kg km}^{-2}$). Thus even in the peripheral area there is probably an adequate food supply and the critical factor in pup survival is the number

of provisioning adults that can capture that prey and make it available to the pups.

Hamilton's theory of inclusive fitness seems particularly relevant to the observed behaviour of helpers⁵. Inclusive fitness consists of: (1) the individual's personal fitness in terms of reproductive success; and (2) the effects of his behaviour on the reproductive fitness of his conspecifics multiplied by their degree of relatedness. 'Inclusive fitness', therefore, examines the lifetime effect of an individual's behaviour on the next generation's gene pool, not only in terms of his own reproduction but also that of related individuals⁶. According to Hamilton an individual's behaviour will be favoured by natural selection if it increases inclusive fitness.

Jackal helpers are as closely related to their full siblings as they would be to their own offspring. As their contributions as helpers increase pup survivorship, their own inclusive fitness is enhanced by kin selection until such time that breeding is attempted. In fact, an adult helper gains more (yield 1 pup per adult) by being a helper of its parents than by finding a mate and raising its own pups aided only by its mate (yield 1/2 pup per adult). Helpers may also derive benefits from extended experience on the home territory in terms of increased survivorship, increased long-term reproductive success and acquisition of a portion of the home territory⁷. Parents benefit from the presence of helpers from increased reproductive success due to higher pup survivorship, and increased inclusive fitness as parents accrue more reproductive benefits by helpers investing in their pups ($r = 1/2$) than by helpers producing their own pups (grandpups $r = 1/4$). Thus, it is in the parent's best interest to retain helpers. The benefits of retaining helpers are potentially limited by available food resources.

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Is intragenic recombination a factor in the maintenance of genetic variation in natural populations?

INTRAGENIC recombination between two different existing alleles in a population can create new alleles. The role of this process in maintaining variation in a natural population has been investigated¹ by assuming that a gene consists of two sites, each of which can mutate to an infinite number of unique 'alleles' (the infinite allele model of Kimura and Crow²). Using this model it was shown that if the product of the population size, N , and the mutation rate, μ , is ≥ 1 , and the recombination rate, r , is the same order of magnitude as the mutation rate, then intragenic recombination significantly increases the number of alleles maintained in a finite population. Moreover, this is not

equivalent to an increase in the mutation rate as the variance of homozygosity and the variance of the number of alleles are larger. This implies that the sampling theory of neutral alleles developed by Ewens³ does not apply to the situation where intragenic recombination is important in maintaining variation⁴. Application of these results to the study of intragenic recombination in natural populations would require a large number of independent samples in order to estimate the variance of homozygosity and the variance of the number of alleles. However, data from natural populations usually consist of the number of alleles and their frequencies in a single sample. Therefore, for the effect of intragenic recombination to be observable, there must be a detectable change in the distribution of the allele frequencies in a sample which contains k alleles. We present here the results of Monte Carlo simulation which show that intragenic recombination in the equilibrium population increases the frequency of the most common allele, increases the number of alleles which occur only once in the sample, and increases the homozygosity.

The Monte Carlo simulation utilises the same algorithm we used previously¹. Briefly, for each of the $2N$ genes of the next generation two genes are selected at random with replacement from the present generation; one of the four alleles, the two recombinants and the two non-recombinants, are then selected with probabilities $r/2$, $r/2$, $(1-r)/2$ and $(1-r)/2$, respectively; and lastly, it is determined whether a mutation occurs at each of the two sites with probability ν where $\mu = 2\nu$. This is equivalent to the model of random drift with two loci⁵. For the simulation of intragenic recombination, the parameter values, $2N = 200$, $4N\mu = 4$, and $r = 10\mu$, were used because it has been shown that there is a significant effect of recombination on the allelic variation maintained in a population with these parameter values¹. A sufficient number of samples, each with k alleles, is obtained in the following manner: each sample is the first generation which contains k alleles and which occurs at least $2N$

Table 1 Statistics of the allelic distributions of 400 samples from Monte Carlo simulation of intragenic recombination

	$r = 0$		$r = 10\mu$	
	Mean	Variance	Mean	Variance
Frequency of the most common allele	0.234	0.00532	0.258	0.00841
Number of singleton alleles	7.255	4.85	7.78	5.63
Homozygosity	0.119	0.00132	0.134	0.00264

$2N = 200$ and $k = 25$.

generations after the previous sample. Samples are collected at least $2N$ generations apart to minimise the effects of co-variance among samples. The results presented are for 400 samples with $k = 25$ and are representative of those obtained for other values of k . For comparison, 400 samples with $k = 25$ alleles are collected in the same manner with the parameter values, $2N = 200$, $4N\mu = 8$, and $r = 0$. $4N\mu = 8$ is a valid choice of the parameter value for the case of no recombination, as Ewens³ has shown that the distribution of allele frequencies conditional on k is independent of the value of $N\mu$. The parameter value, $4N\mu = 4$, is not used because the probability that a generation contains 25 alleles is very small.

Three variables are used to compare the allelic distributions of the two sets of samples: the frequency of the most common allele, the number of alleles which occur only once in the sample, and the homozygosity. The cumulative distribution of the most common allele in each of the two sets of samples is shown in Fig. 1, and also the difference between the two distributions. It is seen that the frequency of the most common allele is greater in the case of intragenic recombination than that without recombination. As shown in Table 1, this difference holds for the means and variances of the frequency of the most common allele, the number of alleles which occur only once, and the homozygosity.

Is this pattern of differences observable in data from natural populations? We have stated previously¹ that the observed intragenic recombination rate at the *rosy* locus in *Drosophila*⁶ (which varies from 1.2×10^{-4} to 7.2×10^{-6}) and the observed number of alleles in samples from natural populations^{7,8} are such that intragenic recombination could have a significant role in the maintenance of variation at this locus. The data of Coyne⁷ and Singh, Lewontin and Felton⁸ have recently been extensively re-analysed by Waterson⁹ and it was found that the frequency of the most common allele is significantly too large in each of the pooled samples. There was also significantly greater homozygosity and the singleton alleles were too frequent. The results of the simulation of intragenic recombination presented here are in the same direction as that observed in these data from natural populations. Therefore, intragenic recombination may be responsible for some of the excess of rare and of common alleles observed in samples from natural populations.

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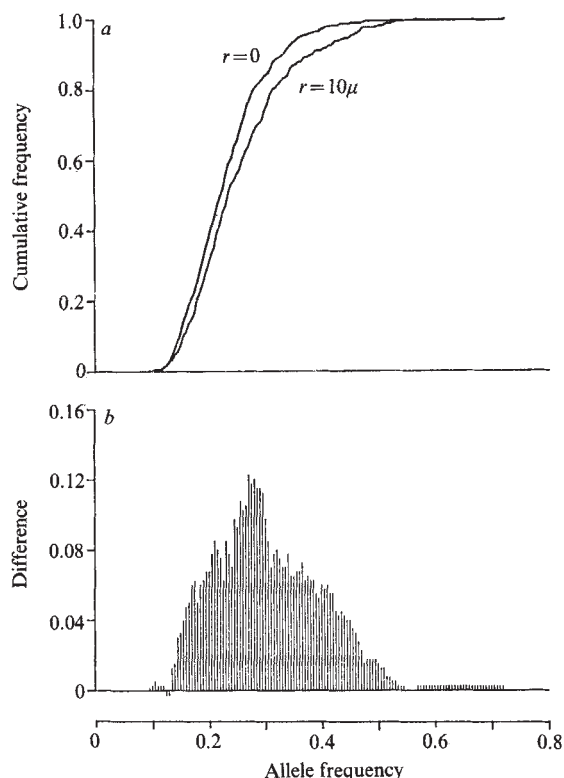


Fig. 1 Cumulative distributions of the frequency of the most common allele from Monte Carlo simulation of intragenic recombination between two sites. For each set of experiments 400 samples were collected, each sample with $k = 25$ alleles, at least $2N = 200$ generations apart. In the case of intragenic recombination, $4N\mu = 4$ and $r = 10\mu$; in the case of no recombination, $4N\mu = 8$. *a*, Cumulative distributions of the frequency of the most common allele; *b*, difference between the cumulative distributions.

Construction of haloaromatics utilising bacteria

LARGE amounts of novel organic compounds are released into the environment by the rapidly growing chemical industry. The main agents for returning natural and, presumably, synthetic organic compounds to the carbon cycle are bacteria and fungi, provided that catabolic enzymes with appropriate specificities, transport systems and regulatory mechanisms can be activated. One or several of these conditions for total degradation do not seem to be fulfilled for certain types of man-made compounds, in particular, the halogenated aromatic hydrocarbons. In this report, we demonstrate the transfer of the TOL plasmid from *Pseudomonas putida* mt-2 (WR 101) to *Pseudomonas* sp. B13 (WR 1) enabling novel strains to be isolated which can utilise

various chlorosubstituted benzoates as their sole source of carbon and energy.

Pseudomonas sp. B13 (WR1) is one of the few bacteria known to be able to use haloaromatics such as 3-chlorobenzoate or 4-chlorophenol as the sole source of carbon and energy^{1,2}. Comparative kinetic studies on the 1,2-dioxygenation of benzoate³, dehydrogenation of dihydrodihydroxybenzoates⁴ and 1,2-dioxygenation of catechols⁵ indicated that the stereo-specificity of the initial enzyme hinders the utilisation of the 2- and 4-chlorobenzoates in addition to 3-chlorobenzoate in this strain. We did, however, demonstrate that the TOL plasmid coded^{6,7} benzoate 1,2-dioxygenase in *Ps. putida* mt-2 (WR101) is considerably less specific and readily co-oxidises chloro-substituted benzoates, including 4-chlorobenzoate³. Nevertheless in contrast to *Ps. sp.* B13, this organism cannot totally degrade halocatechols. Substrate utilisation did not occur, but a number of halosubstituted metabolites of the meta cleavage pathway were accumulated, together with unstoichiometric amounts of chloride (our unpublished work).

Table 1 Construction of halobenzoate-utilising strains

Growth substrate			
3-Chlorobenzoate	3-Chlorobenzoate 3-Methylbenzoate	3-Chlorobenzoate 4-Chlorobenzoate 3-Methylbenzoate	3-Chlorobenzoate 4-Chlorobenzoate 3,5-Dichlorobenzoate
WR1 → WR23	+WR101 (10 ⁻⁵)	WR211	(10 ⁻³) → WR216
WR223 ←	41 °C		
WR1	+WR101 (2 × 10 ⁻⁸)	WR241	(4 × 10 ⁻⁸) → WR941

Donor and recipient cultures were grown for 24 h on nutrient agar plates. The bacteria were suspended in phosphate buffer pH 7.4, 50 mM to 3×10^8 cells ml⁻¹. Portions of each culture were mixed so that the ratio of donor and recipient was 2:1. One ml of the resultant mixture was filtered through 0.2 µm Millipore filter. The filter, after being placed on the surface of nutrient broth agar plates, was incubated for 12 h at 28 °C and then vigorously whirled with 4 ml phosphate buffer to resuspend the cells. The resulting suspension was plated on the appropriate selective medium. The frequency of transfer given in parentheses are defined as the number of exconjugants per donor cell or spontaneous mutation frequency on the selective substrate.

Table 2 Nutritional properties of wild-type and derived strains

Strain designation	Phenotype																	Genotype	Derivation			
	Mcl	Pcl	Dcl	Ptol	Tln	Rib	Seb	41°	Ger	Nic	Pdiol	Eth	But	Pro	Kglu	Man	Test		Hexa	Str	Parent	Treatment
WR1 (<i>Pseudomonas</i> sp. B13, wild-type)	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	s	wt		Dorn <i>et al.</i> ¹
WR23	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	r	str-1	WR1	Spontaneous, selection on nutrient agar plates with streptomycin (0.1 mg ml ⁻¹)
WR101 (<i>Pseudomonas putida</i> mt-2, wild-type)	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	s	wt/TOL		Williams and Murray ⁶
WR211	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	+	+	r	str-1/TOL	WR23	Conjugation with WR101, selection on Ptol/Str agar plates
WR216	+	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	+	+	r	str-1/TOL	WR211	Spontaneous, selection on Pcl agar plates
WR223	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	r	str-1	WR211	Spontaneous, during growth at 41 °C on nutrient broth agar plates
WR241	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	s	/TOL-241	WR1	Conjugation with WR101, selection on Pcl agar plates
WR941	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	s	/TOL-241	WR241	Spontaneous, selection on Dcl agar plates

Phenotype abbreviations: Mcl, 3-chlorobenzoate; Pcl, 4-chlorobenzoate; Dcl, 3,5-dichlorobenzoate; Ptol, *p*-toluate; Tln, toluene; Rib, D-ribose; Seb, sebacat; 41°, growth at 41 °C on nutrient broth; Ger, geraniol; Nic, nicotinate; Pdiol, propandiol (1,2); Eth, ethanol; But, *n*-butanol; Pro, *n*-propanol; Kglu, 2-ketogluconate; Man, D-mannose; Test, testosterone; Hexa, hexadecane; Str, streptomycin sulphate.

+, Good growth; -, no growth; s, sensitive; r, resistant.

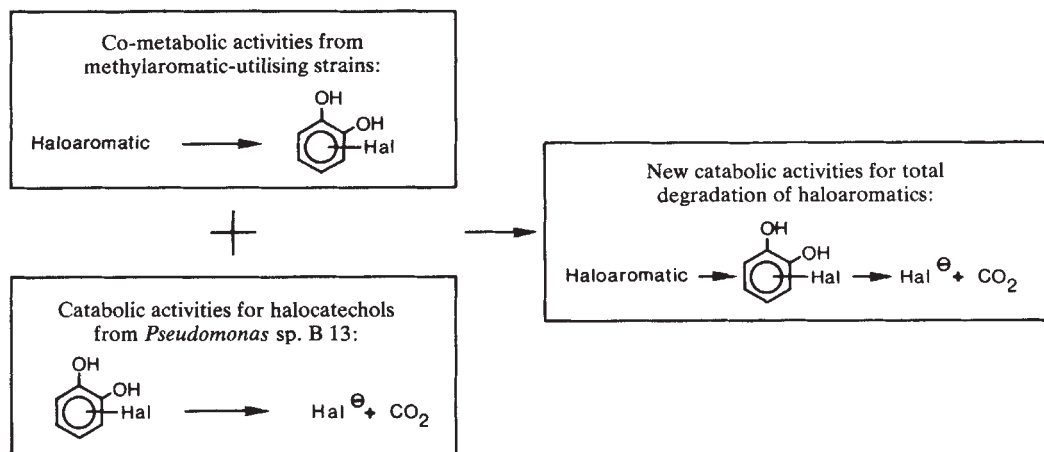


Fig. 1 Concept for the construction of haloaromatics degrading bacteria from methylaromatic-utilising strains and *Pseudomonas* sp. B13 (Hal = halogen).

Exconjugants like WR 241, which is able to grow on 4-chlorobenzoate (see Table 1) were obtained by the Millipore filter technique after selection on 4-chlorobenzoate agar plates. Differentiation by nutritional pattern (Table 2) and analysis of catabolic enzymes (to be published elsewhere) clearly indicated that WR 241 originates from WR 1 as the recipient strain. The ability to utilise 4-chlorobenzoate is the only new property in strain WR 241. Other exconjugants, not listed here, were isolated which could utilise *p*-toluate in addition to 4-chlorobenzoate. From this, it can be deduced that WR 101 functioned as the donor strain. By mating WR 101 with the streptomycin-resistant strain WR 23 of WR 1 and selection on *p*-toluate-streptomycin agar plates, strains of type WR 211 were found which could use both growth substrates of the parent strains, namely, 3-chlorobenzoate and *p*-toluate. By plating WR 211 on 4-chlorobenzoate, spontaneous mutants like WR 216 were isolated which could grow on the new substrate.

Exconjugants, such as WR 241, appeared with almost the same frequency as the product of the frequencies which were observed during selection for strains WR 211 and WR 216. Phenotypic expression for the utilisation of 4-chlorobenzoate seems to require a mutation in addition to the transfer of the TOL plasmid. During growth at 41 °C strain WR 211 can be cured, consequently, WR 223, the resulting strain, lost its ability to grow on *p*-toluate.

Biological devices for the adaptation to novel organic chemicals are mutations in structural genes leading to the evolution of enzymes with novel specificities and activities⁸⁻¹¹ and mutations changing regulatory controls^{12,13}. In another study, the detectable response to stress by novel organic molecules was the hyperproduction of pre-existing enzymes due to gene duplication¹⁴. The active sites of these enzymes possess a slight affinity for compounds of related chemical structure. The second copy enables mutations to accumulate, which are not immediately constrained by selection. An ideal mechanism for accelerated enzymatic adaption to novel substrates is suggested by the observation that the genes for catabolic enzymes are carried on extrachromosomal elements^{15,16}.

In the present study, *Ps.* sp. B13 acquired the ability to utilise novel substrates like 4-chloro- and 3,5-dichlorobenzoate by conjugation with *Ps. putida* mt-2. The initial turnover of the halogenated benzoates was provided by the TOL plasmid-coded enzymes, whereas total degradation and utilisation was accomplished by the halocatechol-degrading enzymes of the recipient strain. In addition, exconjugants were observed in which *Ps. putida* mt-2 seemed to have acquired the genes coding for the enzymes of halocatechol catabolism (our unpublished work). In general, the acquisition of the genes of halocatechol catabolism could overcome accumulation of toxic halocatechols which frequently occurs during co-metabolism of haloaromatics in arene or methylarene-utilising bacteria. We found the same enzymes for total degradation of halocatechols in other pseudomonads which are capable of utilising a variety of haloaromatic

compounds. These had been isolated from continuous adaptation cultures in the presence of *Ps.* sp. B13. The combination of methylarene-utilising strains providing high co-metabolic activities for halosubstituted analogues by their initial enzymes, together with the halocatechol-degrading enzymes of *Ps.* sp. B13, could be a general concept for the construction of new strains (see Fig. 1) which could degrade halosubstituted benzenes, phenols, anilines and naphthalenes, totally.

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Selection of predation-resistant bacteria in continuous culture

VARIOUS models of predator-prey interactions predict the occurrence of reciprocal oscillations in the densities of the predator and its prey¹. Some models predict undamped, neutrally stable oscillations (the Lotka-Volterra model), whereas others predict damped oscillations, the two populations gradually approaching an equilibrium. Few attempts have been made to test these models, possibly because of the rarity of suitable experimental systems. The predatory bacterium *Bdellovibrio*, which preys on other bacteria, provides an ideal system in which predator-prey interactions can be studied in a convenient and accurate way. As part of a study of the ecology of *Bdellovibrio*² we have inoculated bdellovibrios into a continuous culture of prey bacteria growing in a chemostat. The introduction of the bdellovibrios was followed by reciprocal population oscillations.

Table 1 Comparison of *Photobacterium leiognathi* E28 (original strain, O) and two strains (A, B) isolated from the chemostat after 6 days of mixed cultivation with *Bdellovibrio*

	O	A	B
Cell morphology	Rod (pairs)	Rod (pairs)	Oval (in chains)
Colony morphology*	Translucent	Translucent	Opaque
Doubling time in batch culture*	22 min	23 min	36 min
Maxima light intensity (quanta s ⁻¹ ml ⁻¹) in batch culture*	6.0 × 10 ¹¹	8.8 × 10 ¹¹	1.8 × 10 ⁹
<i>Bdellovibrio</i> plating efficiency†	1.0	1.0	<1.0 × 10 ⁻⁷ ‡
t _{1/2} (time to reduce prey population to half original size after mixing with <i>Bdellovibrio</i> at a ratio of 1:10)	10.3 min	10.3 min	>60 min

* On medium MPY (ref. 3).

† Determined as described by Marbach *et al.*³.‡ Macroplaque was obtained when 10⁷ or more *Bdellovibrio* plaque-forming units were placed on the lawn.

However, the oscillations did not persist and were not damped. Instead, a new system evolved in which a *bdellovibrio*-resistant mutant was detected.

The bacterium which served as prey was the luminescent *Photobacterium leiognathi* E28 (ref. 2). It was grown in a chemostat set to hold a carrying capacity of 10⁸ cells ml⁻¹. After the culture reached a steady state, a small inoculum of the predator, marine *Bdellovibrio* BM4 (ref. 3), was introduced.

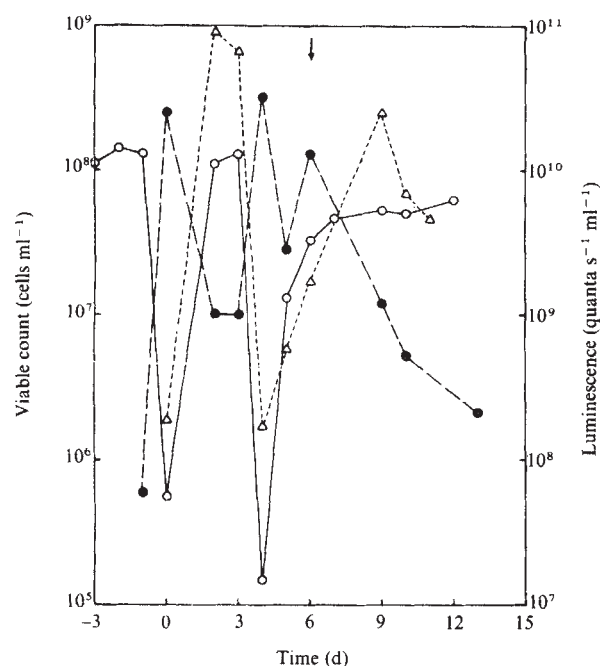


Fig. 1 Growth of *Bdellovibrio* BM4 and *Photobacterium leiognathi* E28 (both strains streptomycin-resistant) in a mixed culture in a chemostat at a dilution rate of 0.1 h⁻¹. The medium used was MPY (ref. 3) diluted 1:15 and supplemented with streptomycin (100 µg ml⁻¹) to reduce the possibility of chance contamination with bacteria. The prey (○) was allowed to reach a steady state, then the flow was stopped and *bdellovibrios* (●) were inoculated and allowed to grow for 24 h in batch culture. At time zero the flow was turned on again and kept constant throughout the experiment. The triangles indicate the light intensity of the culture, measured by a photometer similar to the one described by Mitchell and Hastings⁷. The arrow indicates the first appearance of mutant colonies on the plates. Viable counts were done as described by Marbach *et al.*³, using plates supplemented with streptomycin.

Figure 1 depicts the pattern of growth which followed: classical oscillations are apparent up to the sixth day (in this specific experiment); the pattern then changes, as the prey population which was initially homogenous, begins to show morphologically different types of colonies. From this point the prey population consisted of two different genetic types—one resembling the wild type, having a strong bioluminescence (type A), the other being more opaque, and dark (type B). Single colonies of both types were streaked on agar and isolated into axenic cultures. Figure 2 shows the growth curve and the bioluminescence of the two types isolated from the chemostat, in comparison with the strain with which the chemostat was originally inoculated. Type A grows at a similar growth rate and emits light of a similar intensity to that of the original strain; type B grows at a slower rate and emits light at an intensity less than 1% that of the original strain.

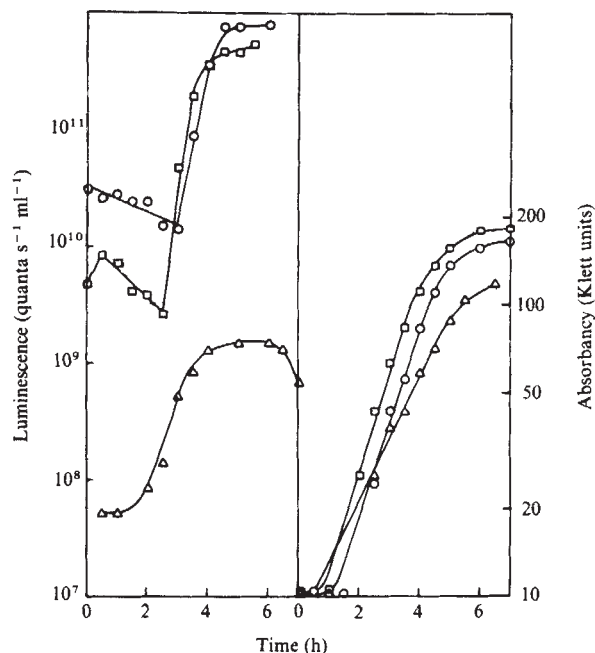


Fig. 2 Growth curve and bioluminescence of *Photobacterium leiognathi* E28 and of the two colony types isolated from the chemostat. □, The original strain inoculated into the chemostat; ○ type A; △, type B. All three cultures were grown in MPY medium in a shaking water bath at 26 °C. Absorbance was measured in a Klett-Summerson photometer using filter no. 42.

When a suspension of each of the two types was mixed with the *bdellovibrios* (at a ratio of 10 *bdellovibrios* per prey bacterium), type A cells were immediately attacked and penetrated, as observed under the phase-contrast microscope, while type B cells remained untouched by the *bdellovibrios*. Death of the type A *photobacteria* followed the attack by the *bdellovibrios* (Table 1).

Plaque assay gave a similar picture: the efficiency of plating of the same *bdellovibrio* suspension on lawns of type B was at least 10⁷ times lower than on type A or on the original strain (Table 1).

In summary, a two-membered culture of a *Bdellovibrio* and a fast-growing susceptible prey evolved into a three-membered culture containing *bdellovibrios*, a prey type similar to the original, and a dark mutant, which grows more slowly and is resistant to the *bdellovibrios*. The different growth rates of the luminous bacterium and its dark mutant would exclude the possibility of their coexistence in the chemostat unless there is an interaction between the two strains. Indeed, when the two were inoculated together in a chemostat operating in the same conditions as the three-membered culture, but without the *bdellovibrios*, type A was maintained in a steady state, while type B was washed out. The resistance of the dark mutant to the *bdellovibrios* would not allow the latter to reproduce so the

coexistence of predator and mutant is also unlikely. However, a mutant with slower growth rate and greater predation resistance may be able to coexist with the wild type and its predator in a stable three-membered system. The stability of such a system has been theoretically predicted by Cramer and May⁴ who, using simple three-species mathematical models, showed that a two prey-one predator system could be stable in circumstances where the two-prey system would in the absence of predation be unstable due to competition.

The possibility of coexistence of a predator and its prey in a homogenous environment has also been questioned⁵. Van den Ende⁶ studying the interaction of a protozoan predator (*Tetrahymena pyriformis*) and its bacterial prey (*Klebsiella aerogenes*) in continuous culture concluded that the coexistence of the two organisms was due to spatial heterogeneity created in the system by the prey bacteria. No such heterogeneity could be observed with the photobacteria, which were homogeneously dispersed in the growth vessel; their persistence could be explained by the presence of the slow growing and predation-resistant mutant.

Although *Bdellovibrio* has been known and studied for 16 years, this is the first report of the isolation of bdellovibrio-resistant mutants. Apparently, conditions prevailing in batch cultures do not allow the evolution of such mutants, while growth in a chemostat in the conditions used in this study repeatedly resulted in their appearance.

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Unusual maternal-fetal blood glucose concentrations in Weddell seal

FETAL whole blood glucose levels are usually lower than, and fluctuate with, maternal levels. That is, a favourable concentration gradient is maintained to allow for the facilitated transfer of glucose across the placenta from mother to fetus^{1,2}. This situation is apparently reversed in the Weddell seal. The concentration of glucose in maternal whole blood is lower than that in fetal blood during at least three metabolic states: in the resting-fasting state, in simulated diving, and in recovery from diving³. We report here that one factor contributing to this unusual relationship between maternal and fetal blood glucose is the relatively low gradient between normal blood levels in fetus and mother. This is made possible by high haematocrit values and a low turnover of glucose in both parent and fetus.

Five pregnant Weddell seals (*Leptonychotes weddelli*) weighing 450–500 kg were captured on the fast ice near Ross Island, Antarctica and taken to the Eklund Biological Laboratory at McMurdo Station. Intermittent blood samples were obtained from indwelling maternal and fetal catheters implanted as previously described⁴. Samples of blood (2 ml) were added directly to perchloric acid and treated as before⁴. Glucose levels in neutralised perchloric acid extracts were determined using the

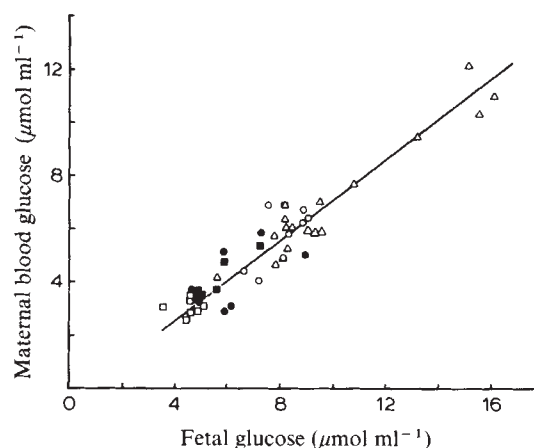


Fig. 1 A plot of glucose concentrations in whole blood of five maternal-fetal pairs. The different symbols refer to different pairs. Blood samples were taken before, during and after simulated diving. The highest glucose values were obtained after intravenous infusion of glucose.

hexokinase assay⁵. In several fetal and maternal extracts, glucose was determined also by the glucose oxidase method, the Benedict assay system and by an automated glucose analyser with a linear response over the range 5–50 $\mu\text{mol ml}^{-1}$. All three assay procedures yielded the same fetal-maternal differences and the same absolute glucose concentrations. To exclude the possibility of the presence of an unusual sugar, whole blood extracts were analysed by gas-liquid chromatography⁶. Glucose was the only major sugar present in maternal and fetal blood; fructose, galactose, mannose, and ribose did not occur in measurable concentrations. The possibility of a significant amount of fructose was also excluded by enzymatic tests using fructose kinase and hexose isomerase to convert fructose to glucose-6-phosphate and then assaying the latter with glucose-6-phosphate dehydrogenase. Establishing the absence of fructose was considered important because of its reported occurrence in cetaceans⁷. Insulin was measured by radioimmunoassay with porcine insulin serving both as the antigen in the preparation of antibodies and as the standard⁸.

Under resting and fasted conditions (in captivity for at least 12 h), maternal whole blood glucose concentrations ranged between 4 and 6 $\mu\text{mol ml}^{-1}$ compared to 5–8 $\mu\text{mol ml}^{-1}$ in fetal whole blood. Similar levels were found in samples drawn from undisturbed seals asleep on ice. Although the fetal concentrations were consistently higher than maternal ones, the two values tended to fluctuate in unison (Fig. 1).

To better understand how maternal and fetal blood glucose levels are regulated, a glucose load (1.5 g per kg of estimated maternal body weight) was infused over a period of 20 min into

Table 1 Fractional distribution of glucose between plasma and red blood cells in the maternal and fetal Weddell seal compared with the sheep

Species	Condition	Glucose ($\mu\text{mol ml}^{-1}$)		Ratio plasma:red cells	
		Whole blood	Plasma	Red cells	
Weddell seal (adult)	Pre-infusion	3.1	7	0.5	14
	60 min postinfusion	13.5	30	2.5	12
Weddell seal (fetus)	Pre-infusion	3.5	6	2.4	2.5
	60 min post-infusion	15.8	22.5	12.9	1.7
Sheep (adult)	Post-prandial		2.5	0.3	8.3
Sheep (fetus)			0.7	0.2	3.5

the maternal circulation of three pregnant seals. In all three animals, the maternal concentration of glucose in both whole blood and plasma rose to values that exceeded those in the fetus throughout the infusion and for a short period after it was completed, but within 20–40 min fetal blood glucose levels were again higher than in the mother (Fig. 2a, b). The plasma concentration of insulin increased sharply in both mother and fetus ~15 min after the rise in glucose levels (Fig. 2b). In the fetus, the concentrations of plasma glucose and insulin followed similar patterns but in the mother, insulin levels remained elevated for at least 5 h after glucose infusion although glucose concentrations were falling.

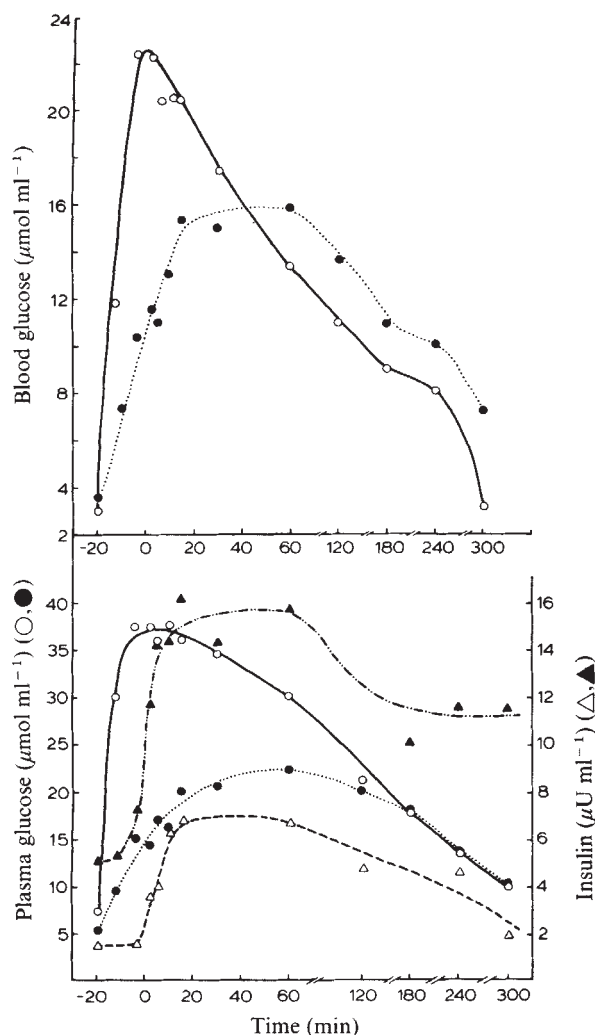


Fig. 2 a, Maternal (○) and fetal (●) concentrations of glucose in whole blood following intravenous infusion of glucose 1.5 g per kg. Time zero indicates the end of the 20 min period of infusion. b, Maternal glucose (○) and insulin (▲) and fetal glucose (●) and insulin (△) concentrations in plasma from the same glucose tolerance test as shown in (a).

As in other mammals, glucose levels are higher in maternal plasma than in fetal plasma and glucose moves from maternal to fetal blood down a concentration gradient. The unusually high glucose content of fetal whole blood relative to the mother arises from several factors. First, the gradient across the placenta is small ($\sim 2 \mu\text{mol ml}^{-1}$) despite the use of arterial samples which would tend to exaggerate the apparent gradient. This is particularly so after glucose infusion when the gradient disappeared for 2 h (Fig. 2b). Second, the ratio of glucose concentrations in red cells to those in plasma in the mother is unusually low whereas that of the fetus is similar to other species (Table 1). Finally, the haematocrit of both mother and fetus is high ($\sim 60\%$ and 70% respectively) which has the effect of increasing the

ratio of plasma: whole blood concentration of glucose, especially in the mother.

Whereas a high haematocrit increases the O_2 carrying capacity of the blood, it clearly reduces the glucose carrying capacity although to a lesser degree in the fetus because of the greater entry of glucose into fetal red cells. The sustained hyperglycaemia after glucose loading (Fig. 2b) suggests that the rate of glucose turnover in the adult seal is low. Likewise, the small gradient of plasma glucose concentrations across the placenta suggests a low turnover rate in the fetus. Thus, neither mother nor fetus is likely to be disadvantaged by having a relatively low carrying capacity for glucose in the blood.

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Immunisation against gametes and asexual erythrocytic stages of a rodent malaria parasite

INVESTIGATIONS into the feasibility of vaccinating against malaria have centred mainly on the use of the sporozoite^{1,2} or the asexual blood stages^{3–6}. Immunisation using sexual stages of malaria parasites has been demonstrated with *Plasmodium gallinaceum* in chickens^{7,8}. Gamete vaccines have been used to produce an effective immunity against transmission of the avian malaria parasite, with little or no effect on its asexual erythrocytic stages. More recently, this study has been extended to *P. knowlesi* infections in rhesus monkeys⁹; transmission-blocking immunity and resistance to asexual infection were obtained, but were fully effective only when the vaccine was combined with Freund's complete adjuvant. We report here successful immunisation of mice against the sexual stages of *P. yoelii* using formalin-fixed gametes. In addition to very effective transmission-blocking immunity, the vaccinated mice were strongly protected against asexual erythrocytic stages of the parasite.

Eight-week-old female outbred Theiler's Original (TO) mice were used as vertebrate hosts. The parasite, *P. yoelii nigeriensis*, was cryopreserved (stabilate reference LUMP 1316) soon after cyclical transmission through the vector *Anopheles stephensi*. Parasites from the first passage from the vector were used for initiating an infection.

P. yoelii preferentially invades reticulocytes. Mice were made anaemic with three subcutaneous injections of phenylhydrazine and infected intravenously (i.v.) with parasitised red cells. As a result of the induced reticulocytosis, the parasitaemia rose to 70–80% with 0.5–1.5% of gametocytes. Mice were bled by cardiac puncture into 50 volumes of a suspended animation (SA) solution (0.21% Tris, 0.96% NaCl and 0.2% glucose, adjusted to pH 7.4) in which gametogenesis was reversibly suppressed¹⁰. The suspension was centrifuged at 500g for 10 min and the red cells were resuspended in a gamete-releasing medium⁸ (2.5 vol of 5% NaCl, 2.5 vol of 10% glucose, 20 vol of 1.46% NaHCO₃ and 100 vol of inactivated fetal calf serum, adjusted to pH 8.0) in a proportion of 1:3 v/v, and the pH readjusted to 8.0. After 30 min at room temperature gametogenesis was complete and the suspension was centrifuged at 500g for 10 min. This procedure was repeated four times, resuspending the packed material in SA solution, so as to recover as many free gametes as possible. The pooled supernatants were centrifuged at 18,000g for 20 min and the pellet containing gametes was suspended in a solution of 1.0% formalin for 30 min at room temperature. The gametes were washed three times in SA solution at 18,000g. After the final wash, counts were made on a haemocytometer using a phase contrast microscope, and adjusted such that 0.2 ml contained 2×10^7 male gametes.

The vaccine contained, in addition to male and female gametes, asexual parasites which under the light microscope appeared to be free of their host red cells, and occasional red cell 'ghosts' and white cells. 0.2 ml of the vaccine contained 2×10^7

Table 1 Oocyst production in mosquitoes fed on vaccinated and unvaccinated mice

Day	Unvaccinated mice			Vaccinated mice	
	No. of oocysts per 10 mosquitoes	Mean no. of oocysts per mosquito		No. of oocysts per 10 mosquitoes	
	1	Expt 2	3	Expts 1, 2 and 3	
1	0	0	0	0	0
2	114	1,638	2,162	130.4	0
3	496	498	1,287	76.0	0
4	1,406	40	0	48.2	0
5	0	62	0	2.0	0
Total counts	2,016	2,238	3,449		

In each experiment, 3 vaccinated and 3 unvaccinated mice were given a blood-induced challenge infection. Batches of mosquitoes were fed on each group of mice on days 1–6 of the infection. The mosquitoes were maintained at 24 °C and oocyst counts were done 1 week later.

male gametes, $6-8 \times 10^6$ female gametes and 10^5-10^6 asexual parasites. 2×10^7 male gametes were obtained from 5.0 ml of infected blood. Each mouse received three doses of the freshly prepared vaccine i.v. at weekly intervals, that is, a total dose of 6×10^7 male gametes. One week after the final dose of the vaccine, vaccinated mice and an equal number of age-matched control mice were inoculated i.v. with 2×10^5 parasitised red cells. Thin blood films were made daily, stained with Giemsa and the parasitaemia and gametocytaemia ascertained. On days 1–6 after challenge, mosquitoes were fed on vaccinated and control mice, one cage per group. Any unfed female mosquitoes were removed from the cages and 1 week after feeding, 10 mosquitoes from each cage were dissected and the midguts examined microscopically for oocysts.

Results were obtained from three separate experiments involving nine vaccinated and an equal number of control animals. The infection was fatal in all but one of the control mice. Death of the control mice occurred either on days 6 and 7, with parasitaemias of 20–30% (Fig. 1a) or on days 18 and 19, with peak parasitaemias of 50–60%. In contrast, the vaccinated mice were apparently aparasitaemic by day 12, with an average clearance time of 8.8 d. The highest parasitaemia recorded in the vaccinated mice was 3.1%, reached on day 4 (Fig. 1a). The average gametocyte counts are represented in Fig. 1b. Mosquitoes fed on control mice had high total oocyst counts, these being derived mainly from feeds taken 2, 3 or 4 d after challenge. Mosquitoes fed on vaccinated mice showed no oocysts at all in their midguts throughout the infection (Table 1).

These experiments demonstrate a very effective immunity which blocks transmission. It does not require the use of an adjuvant and we are now investigating whether fewer gametes and/or smaller numbers of immunising doses can be used; preliminary studies indicate that a single dose can be equally effective. Immunity to asexual erythrocytic stages, though not complete, was also pronounced, manifested by a significant lowering of parasitaemia and a 100% survival as compared to the control mice, in which the parasitaemia was higher and the infection fatal in almost all of the mice. Gwadz and Green⁹ used a gamete/trophozoite antigen mixture in their experiments with *P. knowlesi* and this also induced immunity against asexual parasites. There are two possible explanations. First, contamination of the gamete vaccines with asexual stages might be responsible. Experiments by Playfair *et al.*⁶ and our own experience (unpublished) with saponin-lysed, formalin-fixed asexual stage parasites used as a vaccine without an adjuvant, have resulted in an effective immunity to blood-induced *P. yoelii* infections. The alternative proposition would be that gametes protect against asexual stages because they have protective antigens in common.

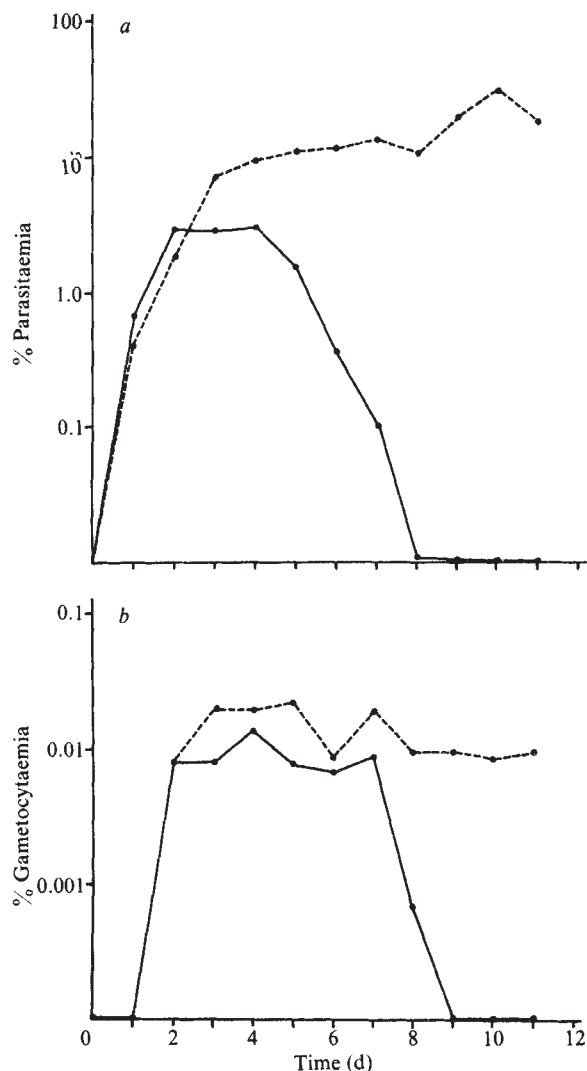


Fig. 1 The course of patent parasitaemia (a) and gametocytaemia (b) in vaccinated (●) and unvaccinated (○) mice.

It is encouraging that such an effective block on transmission is possible by vaccinating with a relative crude antigen preparation, and without the need for an adjuvant. Further improvements both in methods of vaccine preparation and in its presentation are possible.

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Changes in ocular dominance induced in monocularly deprived lambs by stimulation with rotating gratings

AMBLYOPIA in children aged about 6 yr can be rapidly diminished by stimulating the amblyopic eye alone with rotating disks of square-wave gratings¹. Such exposure for four periods each of 7 min has brought many of the patients up to 6/6 acuity, in some cases, after 'patching' of the good eye had been largely ineffective. However, in children of this age, the 'critical period' has probably passed. We decided to study the effects of presenting rotating gratings during the critical period, when the visual system is known to be acutely sensitive to visual deprivation. Could changes in the ocular dominance of cortical neurones be

induced even in the relatively unfavourable conditions of moderate to light anaesthesia? We report here that monocular deprivation of 2-3 d in young lambs results in a marked decrease in the number of cells in the primary visual cortex which can be stimulated through the deprived eye, but that this can be rapidly increased by subsequent brief exposure of the deprived eye to a rotating square-wave grating. We chose to work on lambs and a pygmy goat in which one eye had been closed for a few days shortly after birth because we thought that such treatment would leave few cells driven initially by the deprived eye. As the visual cortex at that time is showing its greatest plasticity, it seemed likely that short exposures to the gratings would then have their greatest effect on the ocular dominance. Others had used repeated stimulation by moving bars on single cells of normal animals, but had obtained rather slight and transient effects². We hoped to obtain reasonable changes in eye dominance during the limits of single acute experiments. Seven animals were used. They had normal binocular visual experience until the 4th day of life and were then monocularly deprived for either 2 (nos 1-4) or 3 (no. 5) d, after which they were anaesthetised and used for the experiment.

In the earlier experiments we anaesthetised the lambs and pygmy goats with 2% halothane followed by intravenous (i.v.) pentobarbitone, 10 mg per kg per h, for surgical anaesthesia, and 0.5-3.0 mg per kg per h pentobarbitone during recording. Although we gave the least pentobarbitone necessary during preparation for full anaesthesia, the blood pressure usually began to fall in these animals within 3-4 h and i.v. dextran was only temporarily effective in raising the blood pressure. The animal tranquilliser acetylpromazine (Crookes) was used to reduce the total dose of barbiturate given, but infusion was often necessary to maintain blood pressure. This had no observable effect on cortical responses and it does not cross the blood-brain barrier. In later experiments we used Althesin (Glaxo) 0.1 ml per kg, i.v., repeated as required, for surgical anaesthesia, followed for recording by 0.5-3.0 mg per kg per h pentobarbitone with 60% O₂, 40% N₂ mixture. The blood pressure in these experiments was well maintained without other assistance, and the cortical responses during the earlier hours of recording were much brisker. These difficulties are peculiar to lambs and kids between 3 and 14 d after birth.

To prevent large sampling errors, we avoided penetrations normal to the surface. Penetrations on the medial side of the ectolateral sulcus gave tracks in V1 of up to 3.5 mm long, usually parallel to the cortical surface. On average we sampled from six

Table 1 Summary of ocular dominance distribution in the seven experimental animals

Animal no.	Days after birth			No. of units in ocular dominance groups 1-7						
	Left eye shut	Mapped		1	2	3	4	5	6	7
1 (L 27)*	4	15	Pre-stimulation	0	0	0	0	1	8	33
			Post-stimulation	5	5	1	2	4	10	28
2 (L 28)	4	22	Pre-stimulation	0	0	0	0	1	5	35
			Post-stimulation	1	4	0	7	3	23	69
3 (PG 2)	4	6	Pre-stimulation	6	0	2	5	9	9	33
			Post-stimulation	6	6	5	7	11	14	16
4 (L 32)*	5	7	Pre-stimulation	3	0	2	5	3	6	27
			Post-stimulation	9	8	9	12	11	19	33
5 (L 33)*	6	8	Pre-stimulation	2	1	1	7	12	14	33
			Post-stimulation	29	6	9	4	2	20	29
6 (L 34)*	7	9	Pre-stimulation	7	2	4	19	4	21	20
			Post-stimulation	15	10	8	8	3	14	8
7 (L 30)	6	8	Pre-control	1	1	0	1	3	9	15
			Post-control	0	2	1	2	1	4	8

A χ^2 test was used to test significance. L, Lamb; PG, pygmy goat.

* The difference between the pre- and post-stimulation is significant at $P \leq 0.01$.

ocular dominance columns for each sample of 60–100 cells both before and after stimulation by the gratings. All ocular dominance distributions seen in animals deprived for 2–3 d during the first week of life give very similar patterns (Table 1). Also, collecting 100 cells takes about 5 h, and there is no statistical difference between the distribution of dominance in the first 50 and the last 50 cells collected, so that neither sampling nor the passage of time disturbs the distribution.

Single unit recordings were obtained from the striate cortex contralateral to the deprived eye, for in a normal animal in this hemisphere the experienced eye would chiefly excite only 20% of the cells (ocular dominance groups 5–7, Fig. 2a). Tungsten-in-glass microelectrodes with impedances 1–5 M at 1,000 Hz were used³. Visual stimuli were hand-held targets moved against a screen 90 cm from the eyes. Unit properties such as orientation specificity (through each eye) and receptive field type were recorded, but we concentrated mainly on ocular dominance, that is, the degree to which a cell could be driven through one eye or the other.

Whether the responses were from visual areas 1 or 2 (V1 or V2) was determined during the experiment from the visuocortical map produced and subsequently verified in histological sections. In these animals the effects of monocular deprivation for 2–3 d are more marked in V1 than in V2, and so we describe only the units in V1. The presence or absence of spontaneously active but undrivable units was also noted.

Except for the control animal (no. 7), the experimental procedure was the same for each of the other four lambs. The deprived eye was opened and a large sample of single units was

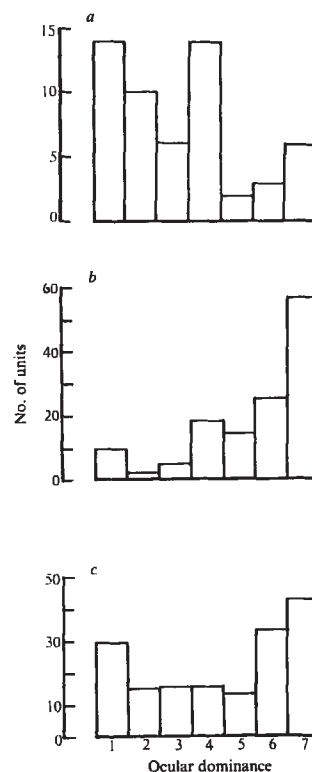


Fig. 2 Ocular dominance histogram of a normal lamb (a) and summed histograms of the four monocularly deprived animals used in the grating experiment and mapped within 10 d of birth; before (b) and after (c) stimulation. The convention of Hubel and Wiesel⁷ is followed: group 1, driven only through the contralateral eye; 2, contralateral eye is much more effective than ipsilateral eye; 3, contralateral eye slightly more effective than ipsilateral eye; 4, the cell can be driven equally through either eye; 5, the ipsilateral eye slightly more effective than the contralateral eye; 6, the ipsilateral eye is much more effective; 7, can be driven only through the ipsilateral eye.

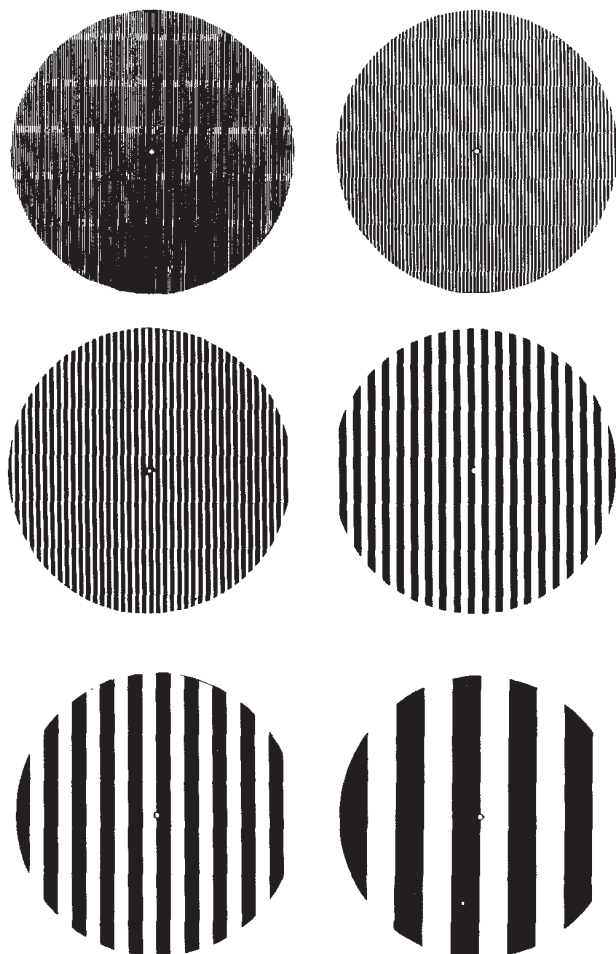


Fig. 1 High-contrast square-wave gratings used in the experiment to stimulate the deprived eye (see text). The spatial frequencies used were 8 cycles deg⁻¹ to 1/4 cycle deg⁻¹.

obtained from V1 during the first few hours. The experienced eye was then closed and the originally deprived eye was stimulated with rotating gratings for periods of 45 to 90 min. Six different spatial frequencies (ranging from 1/4 cycle deg⁻¹ to 8 cycles deg⁻¹, see Fig 1) were presented sequentially in random order during this period, each frequency for about 5 min at a rotation speed of 2 r.p.m.. The whole procedure was then repeated at a faster rotation speed (4–6 r.p.m.). The grating was mounted on a circular disk and subtended at an angle of 35° at the eye. The deprived eye fixated the centre of the grating and was also its axis of rotation. The exact durations of stimulation varied between animals (see Fig. 1 legend).

After grating presentation to the deprived eye, both eyes were closed for about 45 min to allow 'consolidation', as it has been reported that a short period of binocular deprivation may enhance the effects of selective visual experience⁴. Both eyes were then opened again and a new series of penetrations was made to obtain large samples of units. These data were then used for constructing the post-stimulation ocular dominance histograms. A striking shift in ocular dominance was produced by stimulation with rotating gratings. In the stimulated animals there was an increase in responsiveness of cortical cells to stimulation through the deprived eye, most marked in animal 5. These changes remained stable during the post-stimulation recording period (4–6 h).

Two control experiments were also carried out to determine whether the changes observed were specifically due to the presentation of gratings to the deprived eye. In lamb 7, the surgical procedure used was the same as in the other animals, but instead of presenting a rotating grating, the deprived eye was left open for 1 h (with the experienced eye closed) and was exposed to people walking around in the room. The pre- and post-control ocular dominance histograms in this animal were almost identical. Also, from ocular dominance determinations at the beginning and end of 10 h of repeated visual testing of the two eyes, it is clear that mere passage of time under anaesthesia, even with repeated visual testing, does not produce a reappearance of units dominated by the deprived eye.

Lamb 6 was also used for a control experiment. Our sample of neurones from the other four animals indicated the shift of ocular dominance to be expected from 2 d of monocular deprivation; usually about 6–9% of the total number of neurones were chiefly excited through the deprived eye (ocular dominance groups 1, 2 and 3) as compared with 50% in the normal lamb (Fig. 2a). In lamb 6, we opened the deprived eye and closed the experienced eye, and then allowed the animal to walk around the laboratory for 1 h 15 min to allow random visual stimulation of the initially deprived eye before anaesthetising the animal for recording. Several penetrations were then made to obtain a sufficiently large sample of neurones for the ocular dominance distribution.

There was a marked increase in the units driven equally well through either eye (ocular dominance group 4); this requires further investigation. However, the increase in cells dominated by the originally deprived eye was far less than that produced by exposure to the rotating grating for the same period in the other three animals. Hence, random visual stimulation of the deprived eye, even in a conscious animal, may be less effective than a rotating grating. This same animal was then exposed to a rotating grating with only the deprived eye open, and there was a sharp rise in the number of cells driven by the deprived eye.

It is not yet clear whether rotating gratings are the optimal stimuli for producing changes. Experiments on kittens suggest that changes in ocular dominance after reverse suturing begin only after 3 d of visual stimulation⁵, and it would be of interest to replicate our experiments using kittens. However, we were not concerned to find the optimal stimulus to reverse the effects of deprivation, but rather, a rapid way of increasing the proportion of cells driven by the deprived eye. Banks *et al.*¹ found that the effect could be produced in children in whom the critical period had probably ended. In two later experiments in monocularly deprived lambs however, the effect could not be obtained after the end of the critical period. Although the revolving grating increases ocular dominance in our animals and improves visual acuity in patients, this does not imply that the stimulation is acting on the same parts of the visual pathway in both cases.

We therefore conclude that ocular dominance can be changed in periods at least as brief as 1 h, even in an anaesthetised preparation. The mechanism underlying this change in ocular dominance is at present obscure. Experiments are in progress to find out whether the effects of stimulating one portion of the visual field of the deprived eye with a grating may spread to adjacent regions within the same field, and not remain confined to the stimulated area alone. This would tell us whether some central diffuse excitatory mechanism is involved. The preparation would also be useful for studying such pharmacological effects as reported by Kasamatsu and Pettigrew⁶.

Because of its rapidity of onset and long duration, this effect may provide a neurophysiological approach to understanding the nature of some long-term changes in the central nervous system.

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Dexamethasone-induced accumulation of a fibronectin and collagen extracellular matrix in transformed human cells

NUMEROUS differences have been demonstrated between normal and transformed cells in culture. One characteristic finding is the presence of a $2\text{--}2.5 \times 10^5$ molecular weight extracellularly disposed glycoprotein (fibronectin) on normal cells and its absence from cell surface association in transformed cells (see refs 1–3 for reviews). Transformed human cells are known to synthesise and shed fibronectin, although at a much lower rate than comparable normal cells^{4,5}. Studies using immunofluorescence or immunoperoxidase methods have demonstrated fibronectin in pericellular structures of fibroblasts and myoblasts in what we have termed an extracellular filamentous matrix⁶. In transformed cells that are producing fibronectin, though at a lower rate, there is a failure to incorporate the glycoprotein into the extracellular matrix. There is also a similar failure to incorporate collagen into a cellular matrix in transformed fibroblasts⁷. The function of this protein, fibronectin, is thought to be related to cellular adhesiveness and perhaps thereby have some role in cell shape. Transformed cells are less adhesive to each other and to substrates when grown *in vitro* and upon addition of exogenous fibronectin there is a concomitant increase in adhesiveness and change in cell shape^{8,9}. There are many examples of glucocorticoids modulating differentiation some of which include, liver¹⁰, pancreas¹¹, mammary gland¹², retina¹³ and myoblasts¹⁴. Recent work in the exocrine pancreas¹¹ suggests that glucocorticoids may induce specific enzymes without altering the level of general cellular proteins. Glucocorticoids have been shown to stimulate proliferation in growth arrested cells¹⁵ and modify in a synergistic or inhibitory manner cellular responses to other hormones. We report here the effects of dexamethasone on modifying one phenotypic characteristic of cells, namely the occurrence of an extracellular filamentous matrix consisting of fibronectin and collagen. Based on the old hypothesis that transformed or malignant cells are arrested in differentiation, we consider development of the matrix a manifestation of the differentiated state of fibroblasts.

Using the peroxidase–antiperoxidase (unlabelled antibody enzyme) method of immunocytochemical localisation, transformed cells are shown to lack an extracellular filamentous

Table 1 DNA levels of SV40 transformed human fibroblasts

Days of culture	Control (μg DNA per plate)	Dexamethasone (μg DNA per plate)	
		10^{-7} M	10^{-6} M
0	11.06	—	—
1	24.89	25.58	23.51
2	51.86	52.55	48.40
3	99.57	98.88	102.33

Cells were grown as described in Fig. 1 and DNA assay was performed as described previously¹⁴.

matrix which reacts with fibronectin antibodies at either the light (Fig. 1a) or the electron microscopic level (Fig. 1b). Following treatment with 10^{-8} – 10^{-4} M dexamethasone a distinct clustering of fibronectin forms around and adherent to these cells (Fig. 1c, d). This matrix begins to form as early as 24 h after treatment and becomes more pronounced with time in the presence of dexamethasone and increased cell density. The localisation appears most extensive at concentrations of 10^{-7} – 10^{-6} M dexamethasone, whereas at 10^{-9} M, staining intensity drops to control levels where very little staining is seen as on untreated cells. Light and electron micrographs were taken following 3 d of treatment with dexamethasone. Ultrastructurally the pericellular localisation of fibronectin is seen in dexamethasone-treated cultures (Fig. 1d) where none existed in untreated control SV40 transformed human fibroblasts (Fig. 1b). When the specific antibody is adsorbed with fibronectin before reacting with dexamethasone treated cells, no reaction is seen, indicating the specificity of the reaction (Fig. 1e, f).

Using the peroxidase–antiperoxidase method we also observed the failure of transformed cells to produce a collagen matrix as seen at the electron microscopic level (Fig. 2a). Normal cells produce a well defined matrix of collagen that can be detected ultrastructurally with either the unlabelled antibody–enzyme method or ferritin coupled antibody (Furcht *et al.*, in preparation). When the dexamethasone-treated transformed cells (10^{-8} – 10^{-4} M) are examined with immunocytochemistry

using antibodies to procollagen type I (which are known to cross-react with collagen type I) collagen is also observed to form a fibrillar matrix as seen at the electron microscopic level (Fig. 2b). The concordance of the development of a matrix of fibronectin and collagen is quite similar to ultrastructural observations we have made using double antibody labelling at the ultrastructural level on normal human fibroblasts (ref. 16, Furcht *et al.* unpublished).

We were next concerned about the effect of dexamethasone on cell proliferation and examined the effects of dexamethasone on DNA accumulation per day in cultures and found no difference between dexamethasone-treated (10^{-7} – 10^{-6} M) and control transformed cells (Table 1).

A recent study has examined the effects of various hormones on fibronectin of the murine continuous cell line 3T3. The studies of Chen *et al.*¹⁷ show that when murine 3T3 cells are grown in low serum they lose fibronectin-like protein from their surface. It was further suggested that epidermal growth factor (EGF) could specifically induce the formation of a fibronectin-containing matrix on serum depleted 3T3 cells and that this effect was blocked by addition of antibodies to EGF. A variety of other hormones, including cortisol, were also tested which had no effect on fibronectin on 3T3 cells grown in low serum¹⁷. Our data differs in that we very clearly show an accumulation of fibronectin and collagen in transformed human cells treated with dexamethasone.

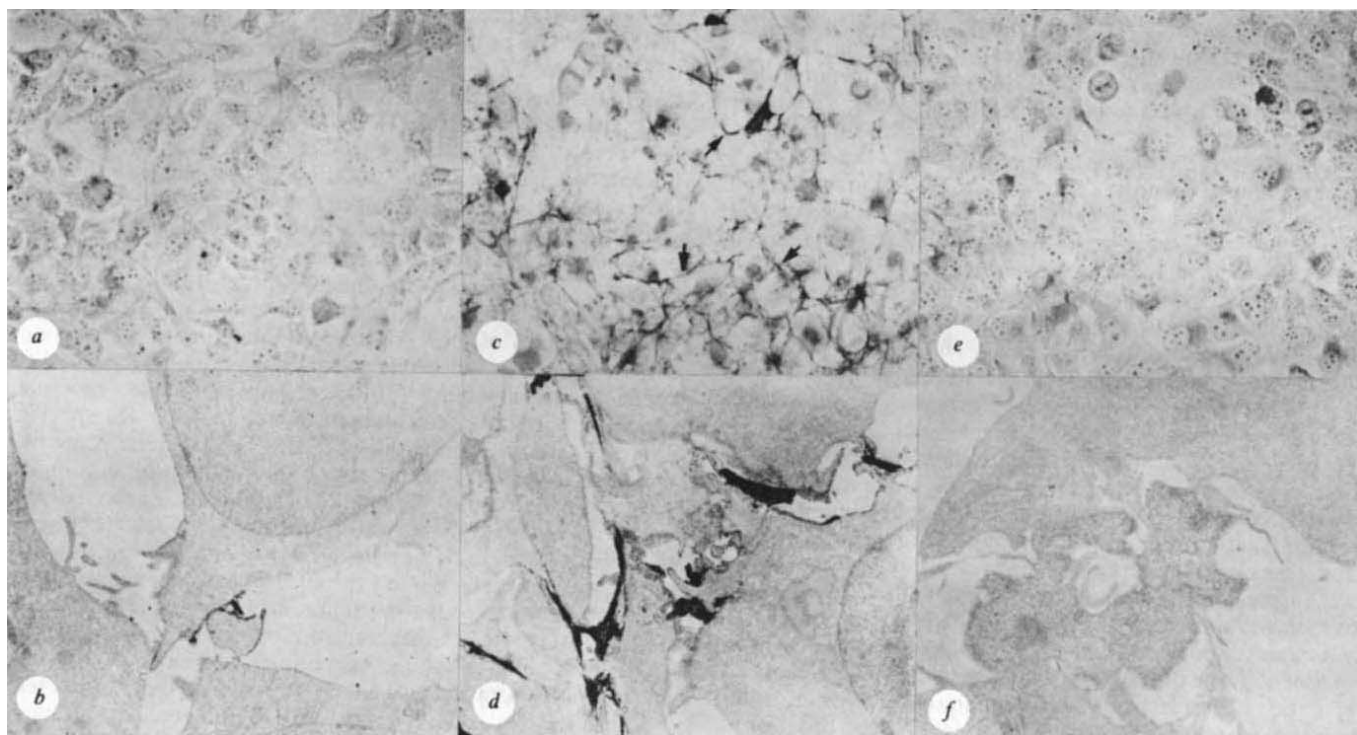


Fig. 1 Light (a,c,e) and electron (b,d,f) micrographs illustrating fibronectin accumulation on dexamethasone-treated SV40 transformed human fibroblasts. Cells are skin fibroblasts from a patient with Fanconi's syndrome which were transformed with SV40. The untransformed counterpart of the Fanconi fibroblasts has a normal distribution of fibronectin and procollagen type I identical to that seen in normal untransformed fibroblasts¹⁶. Cells were plated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum. After one day medium was changed to either regular DMEM with serum supplemented with 10^{-9} – 10^{-4} M dexamethasone. Three days after the medium change samples were fixed with 1% glutaraldehyde (Electron Microscopy Sciences) in phosphate buffered saline for 20 min at 37 °C. Fibronectin and collagen were localised using our modification of the unlabelled antibody–enzyme method described previously⁶. Fibronectin adsorbed antifibronectin was used as the primary antibody for adsorption controls. Adsorption of fibronectin antibody is as described⁶. Results presented are of cells treated with 10^{-7} M dexamethasone. Light micrographs are taken of whole cells grown on Falcon tissue culture dishes after reaction with antibodies and immunocytochemical staining using an Olympus BH microscope equipped with an OM-10 automatic camera. Cells are then embedded *in situ* with Epon, thin sections cut, and viewed without counterstaining in a Philips EM 300 as described⁶. a, No fibronectin localisation is apparent on transformed human fibroblasts grown in DMEM with serum ($\times 130$). b, Ultrastructural fibronectin localisation of transformed cells grown in DMEM with serum. Sparse staining is seen at sites of cell–cell contact and in general is lacking from the cell surface ($\times 3,800$). c, A matrix of fibronectin localisation surrounds cells which have been treated with 10^{-7} M dexamethasone (arrows, $\times 130$). d, A dense staining is seen between cells which were treated with 10^{-7} M dexamethasone ($\times 3,600$). e, No staining is seen if dexamethasone treated cells are reacted with fibronectin adsorbed antifibronectin as the primary antibody ($\times 130$). f, Fibronectin adsorption control. No staining is seen at the ultrastructural level ($\times 3,400$).

It is important to consider a number of points of difference between work by other laboratories and our studies where we use: (1) human cells; (2) transformed cells which do not make fibronectin in a matrix normally; (3) normal culture conditions of medium and serum. This is to be contrasted to the rather artificially induced loss of fibronectin which occurs in serum deprived 3T3 cells¹⁷. As fibronectin is routinely present on 3T3 cells, reproduction of its loss with serum restriction could be subject to numerous difficulties. The cell type of a 3T3 is really unknown with speculation ranging from an endothelial cell¹⁸ to a fibroblast, with also the potential to differentiate into an adipose cell with lipid accumulation (3T3-LI)¹⁹. Furthermore, 3T3 cells will form tumours if they are attached to a substrate and injected into immunosuppressed animals, raising the question of the propriety of using these cells as 'normal' cells²⁰.

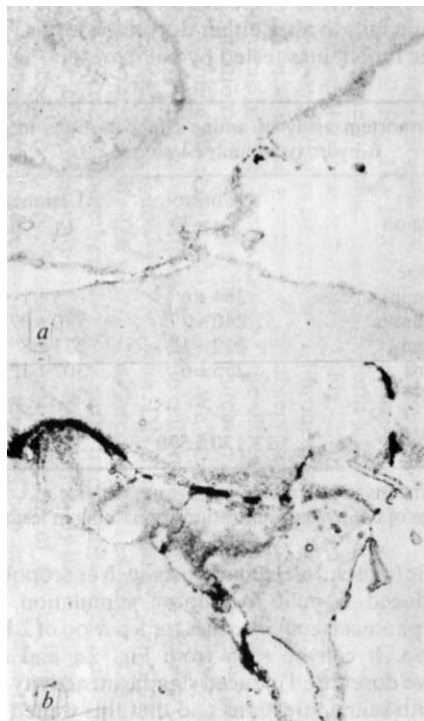


Fig. 2 Electron micrographs of thin sections illustrating immunocytochemical localisation of procollagen type I. Either affinity purified antibodies to procollagen I or the IgG fraction of antiserum to procollagen I was used with similar results. This antiserum also cross-reacts with collagen (Furcht *et al.* submitted). Cells were treated as described in Fig. 1. *a*, Transformed human fibroblasts grown in DMEM lack a collagen matrix ($\times 5400$); *b*, transformed human fibroblasts grown in DMEM supplemented with 10^{-7} M dexamethasone form a collagen matrix identical to the fibronectin matrix seen in Fig. 1*d* ($\times 5,400$).

Several mechanisms should be considered for the dexamethasone stimulated accumulation of fibronectin and collagen associated on the cell surface of transformed human cells. First, recent studies have shown that the translatable mRNA for fibronectin and collagen is decreased in transformed cells²¹ and perhaps dexamethasone is stimulating synthesis of this glycoprotein. Second, dexamethasone may effect whatever the processes are governing the polymerisation of fibronectin and adherence to cells so that more of the fibronectin and collagen synthesised remains or becomes adherent to cells. Based on the known interaction of fibronectin and collagen this might also involve collagen. Third, dexamethasone may modify the breakdown of fibronectin through modulating effects of hormones or proteases. With regard to this, surface associated fibronectin is very sensitive to protease degradation by trypsin and plasmin²². In relationship to this study, many transformed cells have elevated plasminogen activator levels²³ and other

work has shown that dexamethasone will inhibit plasminogen activator levels in macrophages²⁴ and transformed cells²⁵. Lastly, dexamethasone is known to modify prostaglandin biosynthesis²⁶, possibly by release of their precursor, arachidonic acid, or by some mechanism including transcription and translation. Therefore, prostaglandins in some unspecified way may be related to the dexamethasone-induced accumulation of fibronectin on transformed human cells.

In summary, if one postulates that the presence of fibronectin and collagen in normal substrate-attached fibroblast cells represents the differentiated state then the absence with transformation represents an arrest or blockade of differentiation. Based on this postulate it would then appear that dexamethasone treatment of transformed cells is capable of overcoming this arrest of differentiation as manifested in these two phenotypic traits, and results in the accumulation of fibronectin and collagen in transformed human cells.

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Possible behavioural function for noradrenaline-acetylcholine interaction in brain

In the peripheral nervous system, acetylcholine (ACh) acts on presynaptic muscarinic receptors to inhibit the release of noradrenaline (NA) from nerve terminals and by way of nicotinic presynaptic receptors to stimulate release¹. A NA-ACh interaction has also been postulated²⁻⁷ to occur in the central nervous system (CNS). Cholinergic agonists decrease the levels of NA in various brain areas^{5,6}, affect the release of NA in *in vitro* preparations^{3,4} and have similar effects *in vivo* as measured by increased metabolites of NA (ref. 2). A reciprocal interaction, that of NA modulating ACh release in the CNS does not seem to occur, according to evidence⁸ available at present. We report here that depletion of forebrain NA by intracerebral injection of the selective neurotoxin 6-hydroxydopamine (6-OHDA) almost completely blocks the catalepsy induced by cholinergic agonists such as pilocarpine and arecoline⁹⁻¹² and enhances the locomotor stimulation caused by the anticholinergic drugs scopolamine and atropine¹³⁻¹⁵. Although these actions have most usually been ascribed to an interaction with dopamine (DA) systems^{16,17}, our data show that functionally significant interactions between ACh and NA systems also occur.

Forebrain NA was depleted by intracerebral injection of the selective catecholamine neurotoxin 6-hydroxydopamine (6-OHDA)¹⁸ into the dorsal bundle in which NA axons course from their cell bodies in the pontine nucleus locus coeruleus, to innervate wide areas of the forebrain such as cortex and hippocampus^{19,20}. The extent of the depletion achieved is shown in Table 1 for a representative group of animals used in the following behavioural studies. Post-mortem assay²¹ of endogenous amines revealed that cortical and hippocampal NA were depleted to less than 5% of control values, and that hypothalamic NA was also severely reduced, but with little effect on brain dopamine or descending NA systems.

The cataleptic effect of cholinergic agonists pilocarpine and arecoline is shown in Fig. 1. Each point represents a different

group of 10 control and 10 lesioned rats, as the effects of arecoline are reported to change with repeated testing¹¹. Catalepsy was measured by placing the animal's forepaws on a horizontal bar 7.5 cm above the ground and timing the period before the animal removed one or both paws to the ground. Saline-injected rats typically remove their paws within 10-15 s (Fig. 1). Both arecoline and pilocarpine induced considerable catalepsy in control animals, with times of up to 50 s or 100 s being required to remove the paws. Such catalepsy was almost totally blocked in the NA-depleted animals. Significant differences²² were found between control and lesioned groups at all doses used, except 5 mg per kg of arecoline, which failed to produce catalepsy even in controls. The catalepsy induced by haloperidol, a dopamine-receptor blocking agent failed to differ at any dose²³ used, in spite of the induction of considerable catalepsy in both control and lesioned groups. This suggests that noradrenergic interaction is specific for cholinergic mechanisms, since the lesion fails to alter either dopamine levels (Table 1) or dopaminergic function as tested by haloperidol (Fig. 1).

Table 1 Postmortem assay of amine concentrations in control and 6-hydroxydopamine lesioned rats

Region	Control (n = 5)	Lesioned (n = 5)	%*
Noradrenaline			
Hippocampus-cortex	264 ± 6	6 ± 1	2
Hypothalamus	2,240 ± 77	590 ± 87	26
Cerebellum	219 ± 12	271 ± 8	124
Spinal cord	255 ± 6	307 ± 12	120
Dopamine			
Striatum	13,170 ± 570	11,570 ± 1,190	88

Values are means in ng amine per g tissue, with s.e.m.

* Percentage of control concentrations remaining in lesioned tissues.

Cholinergic (muscarinic) antagonists such as scopolamine and atropine induced a mild locomotor stimulation. This was measured in photocell activity cages for a period of 2 h following drug injection. It can be seen from Fig. 2a and b that the representative doses used induced significant activity in controls compared with saline injections and that this waned with time. Although the initial locomotor stimulation over the first hour did not differ between controls and lesioned rats, an enhancement of the stimulant effect was evident in the second hour of testing. This was a central effect, since methylscopolamine, which does not cross the blood-brain barrier, failed to induce locomotor activity even in controls (Fig. 2c). Again, the noradrenergic interaction is specific to cholinergic systems, as the indirect dopamine agonist *d*-amphetamine, which is known to act through dopamine systems to cause locomotor activity^{16,17}, did not differ between control and lesioned groups (Fig. 2d).

The results suggest that cholinergic drugs modulate the activity of a noradrenergic system the function of which may be to influence the degree of behavioural arousal, as reflected by catalepsy at one extreme and locomotor stimulation at the other. Based on changes in sleep-wakefulness after 6-OHDA lesions, similar suggestions regarding the function of ascending noradrenergic neurones have been made by Lidbrink^{24,25}. These behavioural findings also supplement biochemical observations that ACh can increase the release of NA in the CNS²⁻⁷.

We tentatively propose the following model for the NA-ACh interaction. There is evidence for excitatory muscarinic receptors on noradrenergic cell bodies in the brain stem. It is known from microiontophoretic studies that the locus coeruleus has excitatory ACh receptors on its cell bodies²⁶, and may receive cholinergic input from neighbouring cell groups²⁷⁻²⁹. Cholinergic stimulation in the vicinity of the locus coeruleus has also been found to induce atonia and catalepsy³⁰⁻³². In our model, the increased terminal release of NA that would result from the

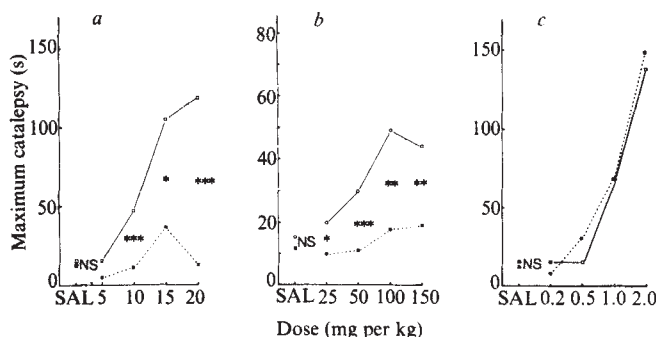


Fig. 1 Catalepsy induced in control and 6-hydroxydopamine lesioned rats. 4 µg 6-OHDA base (Regis, 6-OHDA HBr) dissolved in 2 µl of 0.9% saline with 0.2 mg ml⁻¹ ascorbic acid, was infused through a 34 gauge cannula bilaterally at AP +2.6 mm, ML ± 1.1 mm and DV + 3.7 mm from interaural zero, with the skull level between lambda and bregma. Controls received infusion of saline-ascorbic vehicle. Each point represents the mean of 10 control (□) or 10 lesioned (■) rats and fresh groups of animals were used for different points on the dose-response curve of a given drug. a, Arecoline; b, pilocarpine; c, haloperidol. The drugs were dissolved in 0.9% saline and injected intraperitoneally (i.p.). In the case of pilocarpine, 1 mg per kg methylscopolamine was given i.p. 30 min before the pilocarpine, to block the peripheral effects of the drug which interfere with catalepsy measurement. Control and lesioned groups differed at the following levels of significance using a two-tailed Mann-Whitney *U* test, *5%, **1%, ***0.1%, NS, not significant.

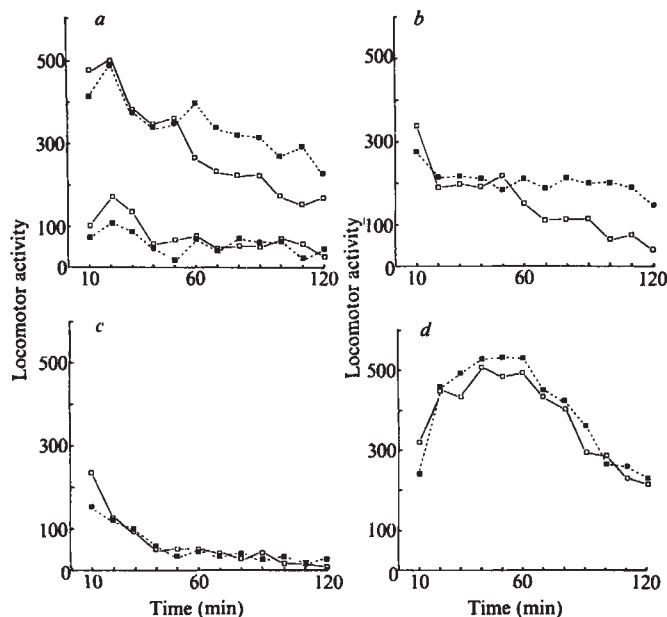


Fig. 2 Locomotor stimulant effect induced in control and 6-hydroxydopamine lesioned rats. Values are mean photocell beam interruptions per 10 min for 10 controls ($\square$) and 10 lesioned ($\blacksquare$) rats. *a*, 1 mg per kg scopolamine (upper lines), saline (lower lines). *b*, 10 mg per kg atropine. *c*, 1 mg per kg methylscopolamine; *d*, 1 mg per kg amphetamine. All drugs were dissolved in 0.9% saline and injected i.p. following 1 h of habituation to the photocell cages. Control and lesioned rats were compared statistically by a repeated measures analysis of variance.

cholinergic stimulation of the NA perikarya is a critical factor in producing the cataleptic response. Conversely, muscarinic antagonists would block the endogenous cholinergic activation of the NA cell bodies, thereby reducing the normal NA release, resulting in motor stimulation; a behavioural state opposite to catalepsy. The model might predict that dorsal bundle lesions would, by themselves, increase locomotor activity. That they do not, suggests that the anti-cholinergic drugs increase locomotor activity by actions on other neuronal systems in addition to the ascending NA projections. That is, rather than being due to decreased NA release alone, the locomotor stimulation induced by anticholinergic drugs seems to be a combined result of decreased NA release and actions on other, presently unspecified, systems. Similarly, although the ascending NA projections seem to play an essential part in muscarinic agonist-induced catalepsy, it is likely that concomitant cholinergic stimulation of other neuronal systems also contributes to this behaviour.

Our results demonstrate for the first time the existence of functionally important interactions between central cholinergic and noradrenergic mechanisms, suggest a role of the noradrenergic system itself and delineate a possible locus of this interaction.

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Reduction in benzodiazepine receptors associated with Purkinje cell degeneration in 'nervous' mutant mice

HIGH affinity benzodiazepine binding sites which are both stereospecific and saturable^{1–3} have recently been characterised in the mammalian central nervous system (CNS). The good correlations obtained between the binding affinities of a series of benzodiazepines and their potencies as anticonvulsants, anxiolytics and muscle relaxants strongly suggest that these sites may function as pharmacological receptors for the benzodiazepines^{1,4}. Although both the gross anatomical^{3,5} and sub-cellular distribution⁶ of these sites have been described, the cellular localisation of these sites is not known, nor has a direct functional link been established between benzodiazepine binding sites and the neuropharmacological effects of these drugs. We now report that 'nervous' mutant mice, which undergo a selective loss of more than 90% of their cerebellar Purkinje cells during maturation^{7,8}, have a marked reduction in the number of cerebellar benzodiazepine receptors. This loss in benzodiazepine receptors seems to be confined to the cerebellum, and is not accompanied by an alteration in the affinity of the receptor for diazepam. These data clearly demonstrate the loss of benzodiazepine receptors in a cell type which has been shown to be both neurochemically⁹ and electrophysiologically¹⁰ sensitive to benzodiazepines, thus providing evidence that benzodiazepine receptors function in the pharmacological actions of these drugs.

Nervous (*nr/nr*), littermate (*nr/+*), and normal (*+/+*) mice of the C3HeB/FeJ strain (Jackson Laboratories), 9–11 weeks old, were used in these studies. Nervous mice were easily distinguished from littermates by a smaller size and characteristic ataxic gait. In addition, cerebellar weights were significantly reduced in the nervous mice when compared to littermate controls or normal mice (36 ± 2.5 mg compared with 55 ± 5.7 and 56 ± 5.0 , respectively). No differences in forebrain weight were noted among the three groups. Mice were killed by decapitation, and the forebrains and cerebella dissected, weighed, and homogenised in 9 volumes of ice-cold Tris buffer (50 mM, pH 7.4). Incubation mixtures consisted of 40 μ l of the 10% (w/v) tissue homogenate, 360 μ l of Tris buffer, 50 μ l of ³H-diazepam (79.8 Ci mmol⁻¹, NEN, diluted in Tris buffer), and 50 μ l of water or unlabelled diazepam (final concentration 3 μ M). Nonspecific binding, defined as the amount of ³H-diazepam binding to tissue in the presence of 3 μ M diazepam, was less than 10% total binding using concentrations of diazepam near the K_d (compare refs 1, 11). Incubation conditions and

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Table 1 Characteristics of ^3H -diazepam binding in 'nervous' (*nr/nr*), littermate (*+ /nr*), and control (*+ /+*) C3HeB/FeJ mice

	^3H -diazepam binding* (fmol per mg protein)		K_d (nM)		$B_{\max}$ (fmol per mg protein)	
	Cerebellum	Forebrain	Cerebellum	Forebrain	Cerebellum	Forebrain
Control (<i>+ /+</i>)	415 $\pm$ 10 (4)	424 $\pm$ 16 (4)	4.4	4.6	957	1,110
Littermate (<i>+ /nr</i>)	398 $\pm$ 12 (4)	440 $\pm$ 21 (4)	4.9	4.4	979	1,023
Nervous (<i>nr/nr</i>)	295 $\pm$ 28 (4) [†]	390 $\pm$ 24 (4)	3.9	4.5	668	1,075

Values are means $\pm$ s.e.m. with the number of animals in parentheses. Values for the K_d and $B_{\max}$ were obtained from the pooled tissue of seven mice per group.

* Binding obtained with 3.5 nM ^3H -diazepam.

[†] $P < 0.02$ compared to littermates, $P < 0.01$ compared to control mice.

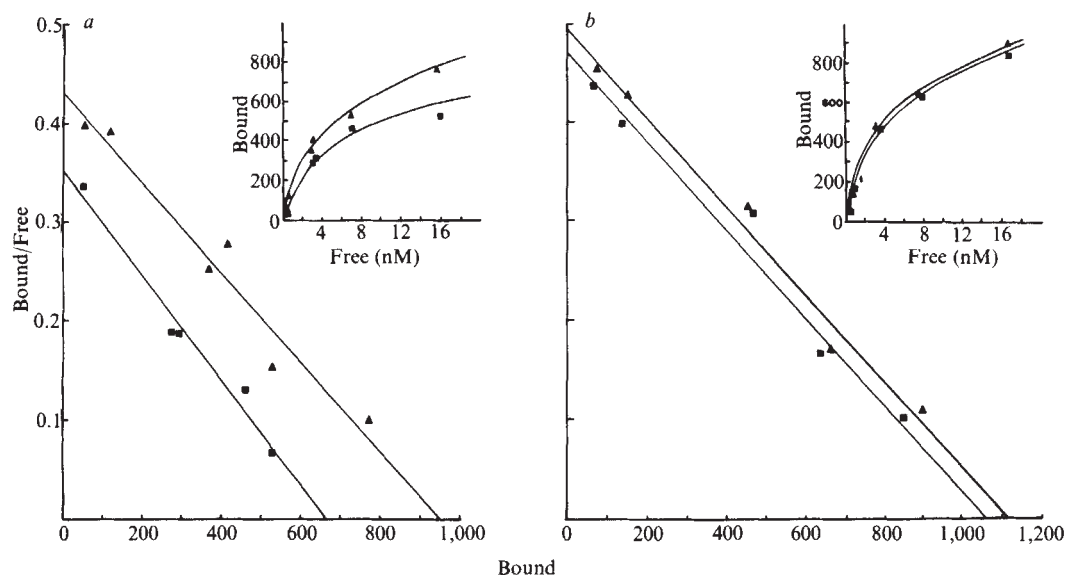


Fig. 1 Kinetics of ^3H -diazepam binding in nervous, littermate, and control mice: Scatchard analyses of binding in nervous ($\blacksquare$) and control mice ($\blacktriangle$) in *a*, cerebellar and *b*, forebrain homogenates. Insets: saturation plot. Scatchard and saturation analysis of littermate (*nr/+*) ($\blacksquare$) and control (*+ /+*) ($\blacktriangle$) mice resulted in nearly superimposable curves (compare Table 1).

materials used for determination of ^3H -diazepam binding have been described elsewhere^{2,11}.

Preliminary experiments were performed using 3.5 nM ^3H -diazepam, as this ligand concentration would reveal differences in either receptor affinity or number. The binding of ^3H -diazepam in cerebella of nervous mice was reduced 25 and 29% when compared with littermate and normal C3HeB/FeJ mice ($P < 0.02$ and < 0.01 , respectively) (Table 1). In contrast, no significant changes in ^3H -diazepam binding were observed in forebrain homogenates among the three groups (Table 1). Scatchard analysis (Table 1, Fig. 1*a*) of ^3H -diazepam binding revealed a marked reduction in the maximum number of benzodiazepine binding sites ($B_{\max}$) in nervous mutant cerebella, with no apparent alteration in receptor affinity for ^3H -diazepam (K_d) (Table 1, Fig. 1*a*). Both the $B_{\max}$ and K_d for ^3H -diazepam binding were nearly identical among the three groups of mice in forebrain homogenates (Table 1, Fig. 1*b*). The reduction in benzodiazepine receptors observed in the nervous mice was manifest whether the results were expressed on a wet weight or mg protein basis.

Benzodiazepines have profound neurochemical and electrophysiological¹⁰ effects on cerebellar Purkinje cells, presenting a model to assess the relationship between benzodiazepine receptors and the pharmacological effects of these drugs in a specific cell type.

The dramatic reduction in benzodiazepine receptors observed in the cerebella of nervous mice, which suffer a selective loss of Purkinje cells, clearly identifies these cells as possessing both a large number and high density of receptors, as Purkinje cells represent only a minute fraction of the total cerebellar neurone population. In contrast, 'weaver' mutant mice which undergo a selective loss of cerebellar granule cells, have been found to have no reduction in cerebellar benzodiazepine receptors (S. Snyder, personal communication). Heterozygous littermates (*nr/+*)

have binding characteristics indistinguishable from control animals (*+ /+*), demonstrating the necessity for the phenotypic expression of this defect to be manifest (degeneration of Purkinje cells) for a loss in benzodiazepine receptors to occur. The reduction in benzodiazepine receptors in cerebella of nervous mutant mice represents the first reported degenerative genetic disorder linked to altered numbers of receptors in the CNS. Furthermore, these observations strongly support a direct functional association between benzodiazepine receptors and the pharmacological actions of these drugs in the CNS.

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Opiate antagonist reverses the cardiovascular effects of inhalation anaesthesia

THAT opiate receptors may mediate the action of inhalation anaesthetics is evident from the fact that intravenous injection of naloxone decreases the pain threshold in rats during inhalation anaesthesia^{1,2}. But inhalation anaesthetics have not only analgesic and hypnotic properties, they also cause undesirable depression of the cardiovascular system. To investigate whether opiate receptors mediate the cardiovascular response to inhalation anaesthesia we perfused continuously the cerebro-ventricular system of both conscious and anaesthetised dogs with naloxone. Effects of anaesthesia with and without naloxone perfusion were compared for blood pressure, heart rate and baroreflex activity. The constant perfusion technique was adopted to avoid the drastic transients of naloxone brain concentrations which occur following intravenous naloxone application^{3,4} and which might have masked the naloxone effects in recent reports⁵⁻⁷. We show here that (-)naloxone in contrast to (+)naloxone is capable of antagonising the circulatory effects of halothane anaesthesia from the fourth cerebral ventricle.

Eight dogs were prepared for perfusion of their cerebro-ventricular system under general anaesthesia. For perfusion of the fourth ventricle five dogs were used in whom stainless steel cannulae were permanently implanted, one into the rostral end of the fourth cerebral ventricle (inflow cannula) and another one into the cisterna cerebello-medullaris (outflow cannula) which

drains the cerebral spinal fluid from the fourth ventricle. For perfusion of the third ventricle three other dogs were prepared with two cannulae located in each lateral ventricle. Additionally, both common carotid arteries were exteriorised into skin loops for direct blood pressure recording and also for testing of the carotid sinus reflex response to bilateral clamping of the loop arteries.

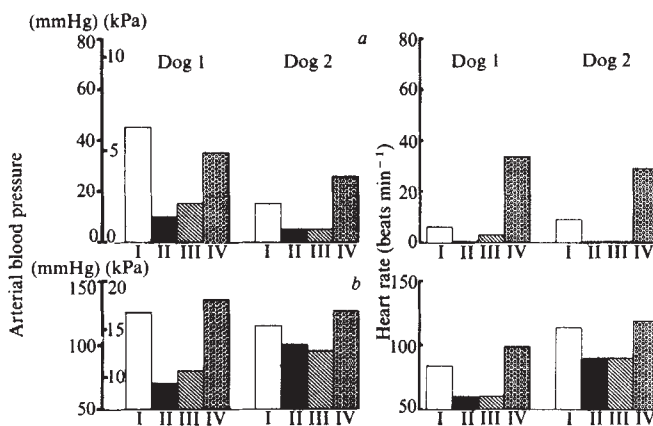


Fig. 2 Comparison of the effects of (+)naloxone and (-)naloxone at a concentration of $20 \mu\text{g ml}^{-1}$ perfused through the fourth cerebral ventricle on halothane-induced arterial hypotension, bradycardia and depressed carotid sinus reflex activity in two dogs. *a*, Response to carotid clamping; *b*, values before clamping; I, conscious; II-IV, halothane anaesthesia 0.72 vol%; II, 60 min; III, (+)naloxone, 20 min; IV, (-)naloxone, 20 min. There is hardly any effect from (+)naloxone but (-)naloxone promptly reverses the halothane effects to levels of the conscious state.

The dogs were trained to lie quietly and were unrestrained in the lateral position for the experiments in the conscious state. The actual experiments were performed 4 weeks after surgery. The animals participated for several months in the study and were familiar with the experimental environment. All the dogs were then repeatedly subjected to the experimental procedure described in the legend to Fig. 1. Ventricular perfusion (0.15 ml min^{-1}) was achieved with a microdrive single-channel push-pull pump (Braun Melsungen Unica B II). The restriction of the perfusion to the respective ventricles was verified by post-mortem dye distribution studies in each dog. The animals were anaesthetised with halothane at concentrations of 0.72-0.82 vol% in pure oxygen. The individual concentration was adjusted to render the animal analgesic as tested by pinching its nose with a haemostat.

Halothane was administered by a calibrated vapouriser adjusted to keep the end-expiratory concentration of the anaesthetic constant, which was verified by mass spectrometry (Perkins-Elmer MGA 1100). The responses of the animals to repeated bilateral carotid clamping of 1 minute duration were evaluated from continuous recordings of the heart rate and the arterial pressure.

In conscious dogs perfusion of the fourth cerebral ventricle with (-)naloxone results in a small increase in blood pressure and heart rate in association with unchanged reflex response of blood pressure but clearly augmented heart rate reflex (Fig. 1, columns I and II). During anaesthesia in combination with ventricular perfusion with Ringer's solution without naloxone (column III) all parameters are conspicuously reduced. But, most important, when (-)naloxone was added to the perfusate all parameters came back to the levels of the conscious controls. In fact, except for the heart rate levels, there are no significant differences present between the naloxone effects at the end of the 20 minute perfusion period (column IV) and the conscious state (column I). Finally, about twice the halothane concentration is necessary to antagonise the naloxone action (column V).

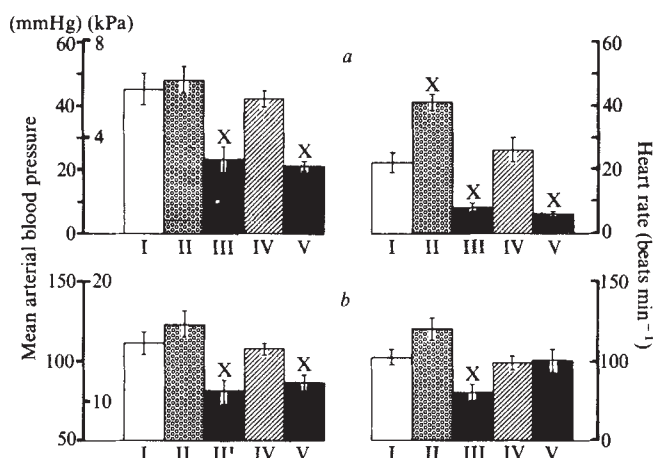


Fig. 1 Effects of continuous perfusion of the fourth cerebral ventricle without and with (-)naloxone at a concentration of $20 \mu\text{g ml}^{-1}$ on blood pressure, heart rate and carotid sinus reflex activity in conscious and anaesthetised (halothane) dogs. Perfusion periods are always 20 min. Halothane anaesthesia in combination with perfusion of the ventricle with Ringer's was maintained for 60 min. *a*, Response to clamping of carotid arteries; *b*, values before clamping. I, Conscious+Ringer, 20 min; II, conscious+naloxone, 20 min; III, anaesthesia 0.75 vol%, 60 min; IV, anaesthesia+naloxone, 20 min; V, anaesthesia 1.37 vol%, 20 min. Averages ($\pm$ s.e.m.) from 10 experiments in three dogs. Differences relative to the controls (column I) were tested with Student paired *t*-test (X, $2 P < 0.005$).

These observations suggest that structures bordering the fourth cerebral ventricle mediate the naloxone effect on inhalation anaesthesia. This is likely as (–)naloxone was found to be completely without influence on the circulatory responses in three dogs in whom the third and lateral cerebral ventricles were perfused under essentially the same protocol. Therefore, possible candidates for sites of interaction with naloxone are those opiate receptors located on the floor of the fourth cerebral ventricle and which continue into the periductal grey matter^{8,10}. These receptors are located rather superficially and opiates bind intensively here¹⁰, penetrating to about 800 μm after local application¹¹. Thus, there are favourable conditions in our experiments for an interaction between naloxone and these receptors. Finally, proof for the specificity of these effects came from observations that (+)naloxone does not reverse the cardiovascular effects of halothane anaesthesia. This was shown in two dogs (Fig. 2) where perfusion of the fourth ventricle with (+)naloxone for 20 min had no discernible effects in the conscious state and did not reverse the arterial hypotension, the bradycardia and the depressed baroreflex activity of halothane anaesthesia maintained for 60 min. In contrast, all circulatory parameters promptly returned to the conscious control levels after a 20 min perfusion period with (–)naloxone.

Thus, it seems safe to conclude that naloxone antagonises the cardiovascular manifestations of inhalation anaesthesia by stereospecific binding with opiate receptors in structures bordering the fourth cerebral ventricle. Possibly, these are the same receptors which are located below the floor of the fourth cerebral ventricle from where opiates and morphinomimetic peptides trigger analgesia^{13–15} and profound sedation with catalepsia^{16,17}.

Whatever the pharmacological mechanism behind the naloxone effect on inhalation anaesthesia might be, our observations strongly suggest that the opiate receptor system seems to be of importance for its mediation. If this is so, then it might be necessary to reconsider the currently held view that inhalation anaesthetics exert their pharmacological effects by nonspecific action on cell membranes. At least the cardiovascular manifestations of halothane anaesthesia are more likely to be the consequence of its interaction with opiate receptors in the brain rather than of nonspecific side-effects on the heart and/or blood vessels.

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Local anaesthetics inhibit tension development and Nile blue fluorescence signals in frog muscle fibres

FLUORESCENT DYES have been increasingly used to measure fluorescence intensity changes associated with changes in transmembrane potential in various preparations^{1,2}. The use of these compounds is of particular interest in muscle fibres, as they can be used to detect potential changes occurring across membranes not directly accessible to electrical measurements, and possibly associated with excitation-contraction coupling processes. For example, muscle fibres stained with Merocyanine 540 and electrically stimulated, give an early fluorescent signal which has been thought to arise from surface and T-system membranes^{3,4}. More interestingly, fibres stained with Nile blue A and electrically stimulated have been reported to give fluorescence signals probably arising from the sarcoplasmic reticulum membrane^{2,5–7}. Optical retardation changes arising from this membrane system have also been reported^{8,9}. These results seem to indicate the existence of an electrical response across the sarcoplasmic reticulum membrane which might be associated with calcium release from these structures during contractile activation. To test this possibility, we investigated and report here the effects of the local anaesthetics, procaine and tetracaine, on tension development and their comparison with the effects on the fluorescence signals generated in muscle

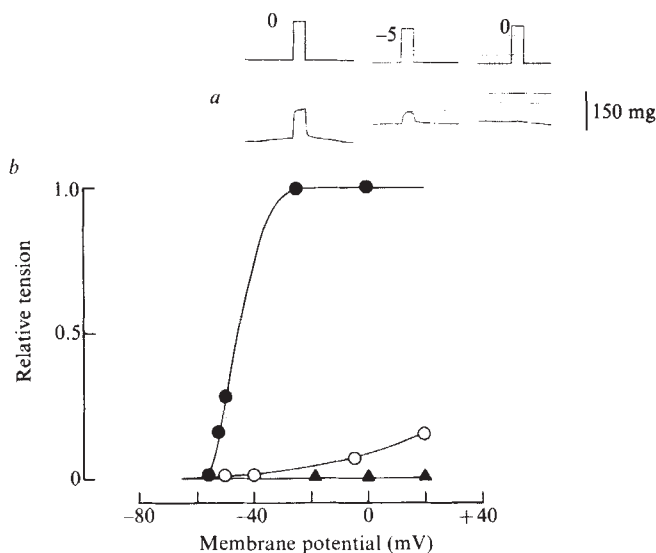


Fig. 1 Effect of tetracaine and procaine on the tension developed by three single fibres of approximately the same diameter (60–70 μm). The experiments used small bundles of short fibres (1–1.5 mm) dissected from the *Lumbricalis brevis* digiti IV of the hindlimb of *Rana pipiens*. A two-microelectrode voltage-clamp technique was used to hold the membrane potential at a value of -100 mV and to apply depolarising pulses¹³. Tension developed by the clamped fibre was registered by means of an RCA 5734 transducer. The fibres were bathed in normal Ringer's solution containing 10^{-6} g ml^{-1} TTX to abolish sodium-dependent regenerative responses and improve the clamping conditions. *a*, From left to right, contractile responses (lower traces) to 1-s depolarising pulses (upper traces) obtained in normal Ringer, and in the presence of 5 mM procaine and 0.25 mM tetracaine, respectively. The level of the membrane potential during the pulse is given. *b*, The tension-voltage relationship obtained with the different pulses on the same fibres. Similar results were obtained with other fibres, although in the case of procaine the effect varied between different fibres. ●, Normal Ringer solution; ○, 5 mM procaine; ▲, 0.25 mM tetracaine.

fibres stained with Nile blue A. Procaine, and to a greater extent tetracaine, are thought to inhibit calcium release from the sarcoplasmic reticulum^{10,11}.

Figure 1 shows the tension-voltage relationships obtained with three different fibres in the absence of local anaesthetics, in the presence of 0.25 mM tetracaine, or in the presence of 5 mM procaine using voltage-clamp depolarising pulses. It can be observed that 0.25 mM tetracaine is a more effective inhibitor of tension development than 5 mM procaine. Furthermore, it is important that with tetracaine (>0.1 mM) tension was abolished in every fibre tested, whereas with procaine the effect varied considerably between fibres. This variability may account for the apparent discrepancy with the work of Thorens and Endo¹².

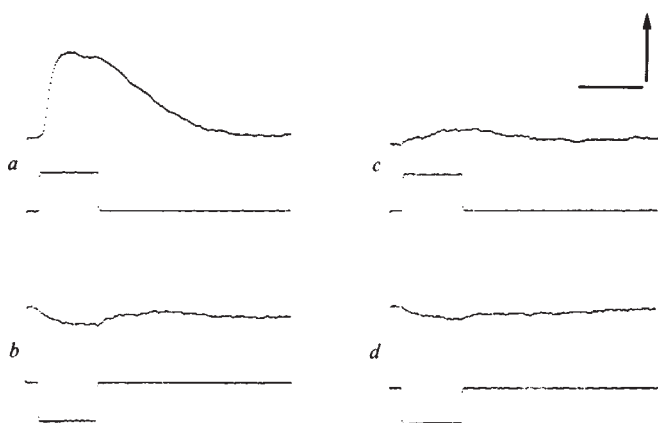


Fig. 2 Effects of tetracaine on the fluorescence signals in a stretched bundle of muscle fibres dissected from the semitendinosus muscle of *Calyptocephaleya gayi*, stained in Ringer plus 0.02 mg ml^{-1} Nile blue A (Allied Chemical). The chamber and optical arrangements were the same as previously described^{4,6}. The bundle was stretched along a trough separated by Vaseline into a narrow central compartment (4 mm wide), where the bundle was illuminated, and two lateral compartments. Fibres in the bundle were depolarised by making the central compartment negative with respect to the lateral ones (positive currents) and hyperpolarised by reversing the polarity. To eliminate tension further and prevent regenerative action potentials, the compartments were filled with hypertonic 345 Tris Ringer solution (345 mM Tris-hydroxymethyl aminomethane-Cl, 1.8 mM CaCl_2 and 2.5 mM KCl). In each section, the upper trace shows the fluorescence signal and the lower trace the current pulse. In a and b, 345 Tris Ringer was used and in c and d 1 mM tetracaine was added to 345 Tris Ringer. a, c Correspond to depolarising current pulses and b, d to hyperpolarising current pulses. The magnitude of the arrow corresponds to a ratio of fluorescence change to basal fluorescence ($\Delta F/F$) equal to 2×10^{-3} for a and b, and 1.5×10^{-3} for c and d. The horizontal calibration bar corresponds to 100 ms. The excitation filter used in the optical record had a central wavelength of 625 nm and the collected light, at right angles, was filtered by a barrier filter that absorbed the excitation light (Schott RG-645). Temperature, 17°C .

Figure 2 demonstrates the effect of tetracaine on the extrinsic fluorescence signal of muscle fibres stained with Nile blue. Fluorescence signals in response to depolarising (Fig. 2a) and hyperpolarising (Fig. 2b) current pulses indicate that, although of the same magnitude, the two pulses induce widely different fluorescence changes. The fluorescence change in response to the depolarising pulse is much the greater, suggesting the presence of a nonlinear membrane potential change. This potential change cannot be due to sodium-dependent regenerative responses, as the external solution was prepared without this cation. Figure 2c,d also shows that 1 mM tetracaine effectively abolishes the large component of the fluorescence change elicited by the depolarising pulses. The remaining signals behave more linearly for depolarising and hyperpolarising pulses, giving positive and negative fluorescence changes, respectively, with time courses similar to those recorded with Merocyanine 540 (ref. 4). The responses to hyperpolarising

pulses are larger, probably due to increased membrane resistance in the presence of tetracaine.

Figure 3 shows the effect of procaine on the fluorescence activation curve. It is clear that in the absence of procaine, this

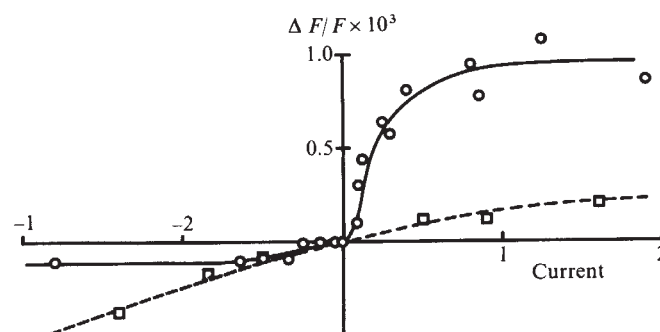


Fig. 3 Effect of procaine on the activation curve of the fluorescence signal. The points were obtained from an experiment in a bundle of fibres dissected from the semitendinosus muscle of *Calyptocephaleya gayi*, the procedure was the same as shown in Fig. 2 except that 10 mM procaine was used instead of tetracaine. The curve is a plot of $\Delta F/F$ against the current intensity of the depolarising and hyperpolarising pulses. The abscissa represents the size of the current pulse in arbitrary units. It must be stressed that the amount of current passed depended on the efficiency of the Vaseline seal between the three compartments (see legend to Fig. 2). The ordinates represent the peak values of the fluorescence signals. Circles correspond to experimental values obtained in 345 Tris Ringer and squares to values recorded in 345 Tris Ringer + 10 mM procaine. Temperature, 17°C .

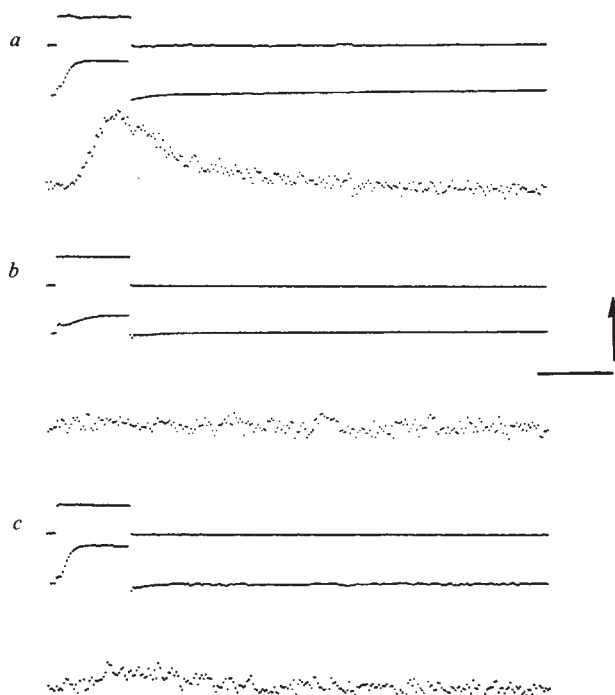


Fig. 4 Effect of tetracaine on the fluorescence signals and delayed rectification of voltage-clamped, cut single muscle fibres. The fibres were dissected from the semitendinosus muscle of *Rana catesbiana*. The techniques and optical arrangement used are described elsewhere⁷ and are based on the work of Hille and Campbell¹⁴. Pool A, (see ref. 14) contained normal Ringer + 10^{-7} TTX. Pools C, B and E contained the following solution: 120 mM K-aspartate; 5 mM ATP- Na_2 ; 1 mM MgCl_2 ; 2 mM EGTA-Tris; pH 7.2. In each section, the upper trace corresponds to voltage, the middle trace to the current, and the lower trace to fluorescence. a, Control records when the portion of the fibre in Pool A was immersed in Ringer + 10^{-7} M TTX. b, Records in Ringer + 10^{-7} M TTX + 1 mM tetracaine. c, Records after 30 min of recovery. The arrow corresponds to a $\Delta F/F$ of 7.2×10^{-3} , a voltage of 200 mV and a current of $13 \mu\text{A}$. The horizontal calibration bar corresponds to 30 ms. Temperature, 20°C .

curve has a steep S-shaped form reminiscent of the tension curve. On the other hand, in the presence of 10 mM procaine, the steep activation of the fluorescence for depolarising current pulses disappears, leaving only the more linear dependence probably arising at the surface and tubular membranes.

Finally, Fig. 4 shows the effect of 1 mM tetracaine on the fluorescence signal and on the membrane current of a single muscle fibre, voltage clamped, using the technique described by Hille and Campbell¹⁴ but with a modified chamber which also allowed optical recording of fluorescence^{6,7}. Figure 4a shows the voltage trace, membrane current and fluorescence trace for a fibre immersed in Ringers and 10⁻⁷ M tetrodotoxin (TTX). The fibre was clamped at about -100 mV and depolarised to 0 mV over 100 ms. It can be seen that tetracaine produced a complete blockage of the fluorescence signal. No linear component is observed, probably due to the small signal to noise ratio of the single fibre preparation. Figure 4c shows some recovery after 1 mM tetracaine was removed. Better recovery was observed when lower concentrations of tetracaine were used. The delayed current was blocked by the presence of tetracaine (Fig. 4b) and shows a good recovery on its removal (Fig. 4c). This current corresponds to the delayed K current whose kinetics are slowed and whose voltage dependence has been shown to be shifted by tetracaine¹⁵. Note that the recovery of the fluorescence signal does not parallel the recovery of this delayed current.

The results of these experiments show conclusively that procaine and tetracaine are effective in reducing or abolishing both tension development and the fluorescence signal in Nile blue A-stained muscle fibres. It has been postulated that this fluorescence signal is the optical manifestation of depolarisation of the membrane of the sarcoplasmic reticulum probably associated with calcium release². Recent work carried out with local anaesthetics indicates that these agents are effective in blocking calcium release acting at the level of one of the last steps in the chain of events involved in excitation-contraction coupling. In fact, Almers and Best¹¹ have shown that tetracaine blocks contraction without affecting the charge movement; this indicates that calcium release can be inhibited one step further than the charge movement which is thought to link the electrical events of the transverse tubular membrane with those occurring at the level of the sarcoplasmic reticulum. The present experiments agree with this hypothesis and support the notion that the extrinsic fluorescence signal in Nile blue-stained muscle fibres may be derived from the sarcoplasmic reticulum membrane² and is associated with calcium release during contractile activation.

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Pelvic structure and nature of reproduction in Multituberculata

RECENTLY prepared skeletons of Mongolian Late Cretaceous multituberculate mammals permit complete reconstruction of the pelvic girdle for the first time; its structure suggests that multituberculates may have been viviparous. Most important for these studies is a specimen of *Kryptobaatar dashzevegi*¹ from the Djadokhta Formation (?late Santonian and/or ?early Campanian), in which both the left and right pelvic halves, including equipubic bones, were preserved undistorted and articulated with part of the vertebral column (Figs 1 and 2). Three of the pelves in the same collection, belonging to three different genera are less complete, but have the same structure as the *Kryptobaatar* pelvis. The previously known multituberculate pelvis of the Paleocene *Eucosmodon*^{2,3} was incomplete and although correctly reconstructed did not show some important details, that are now described.

Hopson⁴ argued that viviparity was one of the last elements in a syndrome of reproductive adaptations to have evolved in mammals. We do not know when viviparity developed in multituberculates, but it should be remembered that the Late Cretaceous multituberculates that have been studied are some 130 Myr younger than their presumable Triassic ancestors⁸. If they were indeed viviparous, such a mode of reproduction in multituberculates was developed independently from that seen in therian mammals.

The sexes of individuals represented by the fossil pelves are unknown. All four pelves are of the same morphological pattern and for the purpose of the description that follows I assume that at least one was female and/or that pelvic sexual dimorphism was not an obvious feature in multituberculates.

As pointed out by Granger and Simpson², multituberculate pelves differ from those of other known mammals in being extremely narrow. The left and right pubes and ischia form a very acute angle and appear V-shaped from behind instead of meeting at an angle of about 180° as in most other mammals. In *Kryptobaatar* the opposite pubes are directed steeply ventromedially and meet at an angle of about 40°; the ischia meet at 45° anteriorly and progressively open up to 80° at the ischial arc. The ventral edges of the opposite ischia and pubes are very strongly fused and together form a prominent ridge. The height of the ridge is about 1 mm in *Kryptobaatar* and its length along the pubes and ischia is 15.2 mm (the bone is missing below the obturator foramen, probably due to damage, and only here has the ridge not been preserved). The greatest length of the pelvis is 32 mm. The posterior part of the pelvis in known multituberculates is strongly bent upwards as seen from the side and the ventral ridge of the fused ischia continues far dorsally which means that the free posterior edges of the ischia are short and the ischial arc is very small. The free edges of the ischia are about 3.8 mm long, and the estimated greatest width of the ischial arc is about 4 mm. The length of the pelvic ridge and degree of fusion indicate that the pelvis was rigid and could not have spread ventrally during parturition.

The nature and dimensions of the pelvic girdle bear on the important question of the size of the offspring or egg in multituberculates and so on their mode of reproduction. The cross sectional area of the passage above the ischial arc depends on the position of the tail, which is high in *Kryptobaatar*. Granger and Simpson³ suggested that *Eucosmodon* had a large iliac-sacral angle of 45° or more. This angle appears to be about 35° in *Kryptobaatar*, leaving a distance of about 1-1.5 mm between the base of the tail and the upper border of the ischial arc. Allowing ~0.2 mm thickness for the soft tissues lining the pelvis, I estimate that if the multituberculates were viviparous, the maximum width of the head in a newborn *Kryptobaatar* could be no more than 3.4 mm. This is about 0.13 the width of the mother's

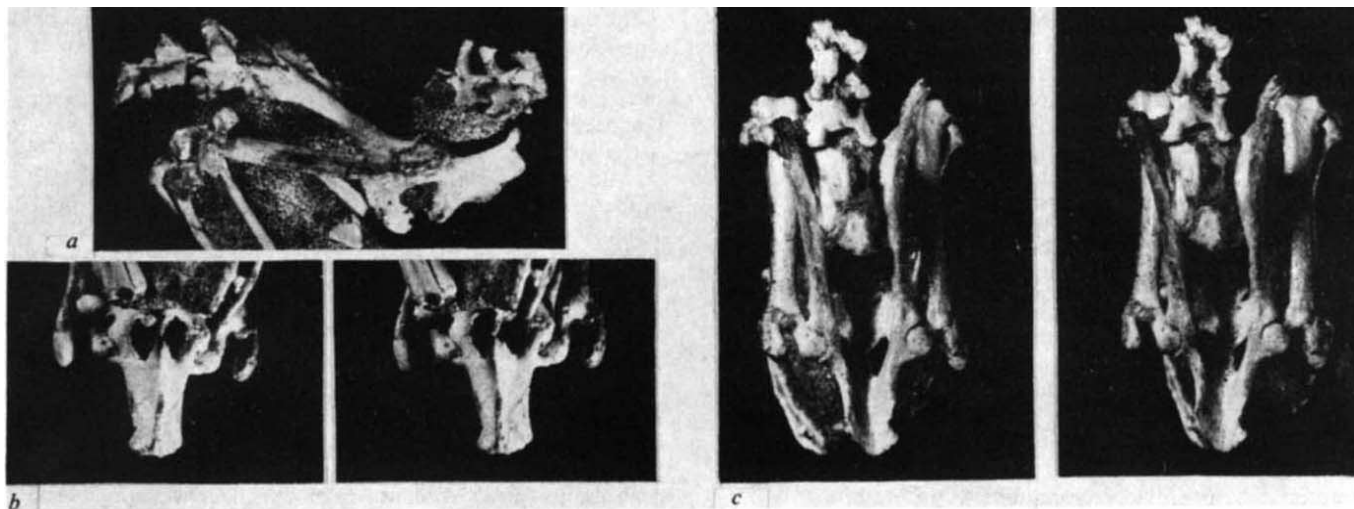


Fig. 1 *Kryptobaatar dashzevegi* pelvis. *a*, Left lateral view, photograph taken before final preparation; *b*, stereophotograph of the same specimen in ventral view, showing the ventral ridge; *c*, stereophotograph of the same specimen in dorsal view, the ischial tuber (present in *a*) was lost during preparation. ZPAL MgM-I/41, all $\times 1.5$.

head compared with a ratio 0.33–0.4 in a rat and 0.45–0.5 in a mouse. There are no measurements available in the literature of the width of the head in newborn marsupials. But all marsupials weigh remarkably little at birth (all less than 1 g), even when the mothers are large⁵.

As in *Kryptobaatar* the profile of the passage within the ischial arc is triangular, the space available for the passage of an egg of circular cross-section would be even less than 3.4 mm diameter. This would mean a smaller egg than any known cleidoic egg. In connection with the egg-laying habitat, the ischial arc in monotremes is wide and U-shaped⁵ and relatively larger and structurally very different from the multituberculate ischial arc. I am therefore of the opinion that multituberculates were not oviparous, but viviparous with an extremely small neonate.

Apart from its relevance to reproductive habitat, the long immobile symphysis (which is relatively longer than in marsupials) and the well developed pelvic ridge may have a bearing to the mode of locomotion in *Kryptobaatar*. Some ungulates (for

example *Antilocarpa* and *Tragulus*) have an immobile symphysis due to the presence of an unpaired interpubic bone and Wegner⁷ argued that this is an adaptation in modern mammals associated with a capacity to jump. Perhaps multituberculates were also able to jump.

Note added in proof: A. K. Lee (Monash Univ.) and L. Selwood (LaTrobe Univ.) have studied a marsupial *Antechinus stuarti* and found the mother's head width to be 15.4 mm, and the two newborn pouch young head widths were 1.6 mm and 1.5 mm, which is about 0.1 of the width of the mother's head (personal communication). This conforms with my estimate of 0.13 for multituberculates.

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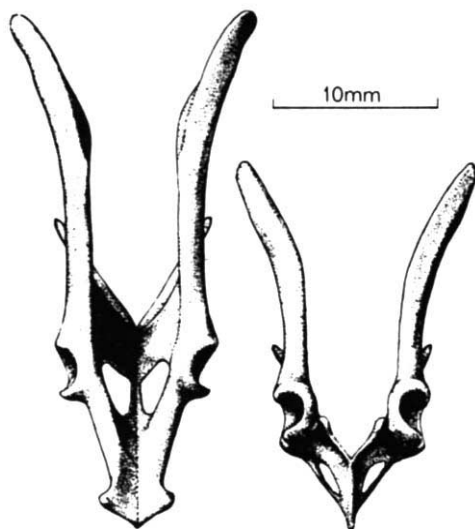


Fig. 2 *Kryptobaatar dashzevegi*. Right, reconstruction of pelvis in dorsal view. Left, reconstruction of the same in posterior view, showing size and shape of ischial arc.

Isolation of the human placenta receptor for epidermal growth factor-urogastrone

EPIDERMAL growth factor-urogastrone (EGF-URO), a single-chain polypeptide of molecular weight $\sim 6,000$, found both in the mouse^{1–3} and in man^{4–7}, is both a potent stimulant of cell proliferation and an inhibitor of gastric acid secretion. Specific membrane receptors for EGF-URO can be detected in various tissues including human placenta^{8–11}. Although the mouse and human polypeptide sequences differ at 16 positions, they can share the same receptor site in human tissues^{3,12}. There has been considerable recent interest in EGF-URO and its receptor, not only because of the potent mitogenic and acid-inhibitory actions of the polypeptide, but also because of the association of viral¹³, chemical¹⁴ and spontaneous¹⁵ cell transformation with a specific reduction in cell receptors for EGF-URO. It is also believed that

EGF-URO and its receptor may be involved in the development, maintenance, and differentiation of tissues as diverse as the lens¹⁶, the palate¹⁷ and the keratin layer of skin¹⁸. In experiments aimed at the isolation of the membrane receptor for EGF-URO, we have observed that, unlike the receptor for insulin¹⁹, but like the muscarinic receptor for acetylcholine²⁰, the EGF-URO receptor loses its ligand recognition property following solubilisation with detergents. We therefore developed an affinity-labelling technique using glutaraldehyde, that can covalently crosslink the receptor with ¹²⁵I-labelled EGF-URO before solubilisation²¹. Independently, Das and coworkers have developed a photoaffinity-labelling technique that can also be used to radiolabel the EGF-URO receptor before solubilisation²². Here we report the success of both our previously described glutaraldehyde crosslinking technique²¹ (using ¹²⁵I-EGF-URO for receptor labelling) and of a newly developed photoaffinity-labelling technique (using a photolabile ¹²⁵I-EGF-URO crosslinking reagent) to identify the EGF-URO receptor in human placenta membranes and to effect the isolation of the affinity-labelled receptor by affinity chromatography using lectin and murine EGF-URO antibody-Agarose columns.

The amino terminus of murine EGF-URO provides the only free primary amine and is the site of the coupling of the photoaffinity label. Because the remainder of the molecule is unchanged, we hypothesised that the anti-EGF-URO antibody would still recognise the EGF-URO moiety after crosslinking it to the putative receptor. The material eluted from the WGA-agarose column was therefore applied to an anti-EGF-URO antibody-Agarose column. Approximately 40% of the applied radioactivity was adsorbed to the column and more than 95% of the protein was unadsorbed. Elution of the antibody-Agarose column with 10% v/v formic acid, yielded the same components, detected autoradiographically, that had been adsorbed by the WGA-agarose column (Fig. 1, columns *f* and *h*). In the experiment depicted (columns *e-h*), the preparation of labelled receptor contained principally band II and a small proportion of band I; both bands I and II were adsorbed by the antibody affinity column. The amount of protein present in the eluate could not be detected (absorbance at 280 nm). When the same amount of WGA-agarose column eluate was applied to an agarose affinity column prepared with pre-immune rabbit serum, >95% of the radioactivity was unadsorbed and the characteristic autoradiographic pattern of the formic acid eluate

Table 1 Affinity chromatography of ¹²⁵I-photoaffinity-labelled EGF receptor

Lectin	Eluting sugar	Radioactivity (%)		Sugar-urea eluate	Residual
		Unadsorbed	Sugar eluate		
WGA	<i>N</i> -Acetylglucosamine	94.8	4.4	0.7	0.1
<i>Lens culinaris</i>	α -Methylmannoside	95.8	3.6	0.4	0.2
Concanavalin A	α -Methylmannoside	93.0	3.0	2.5	1.4
Phytohaemagglutinin	<i>N</i> -Acetylgalactosamine	95.5	1.1	2.0	0.9
<i>Ricinus communis</i>	Galactose	99.7	0.2	0.0	0.0
Peanut Lectin	Galactose	99.8	0.1	—	0.1

The EGF-URO receptor of placenta membranes was labelled using the photoaffinity method as described in Fig. 1. After solubilisation with Nonidet P-40 and clarification by ultracentrifugation, aliquots of the labelled solubilised membranes were applied to 200 μ l lectin-Sepharose 4B columns (5 mg lectin per ml beads) equilibrated with 0.1% Nonidet P-40, 0.2 M NaCl, and 50 mM Tris-HCl, pH 7.5. The columns were washed with 20–30 column volumes of buffer (flow through) and then eluted with an appropriate lectin-specific sugar at 0.2 M. The plant lectin columns were subsequently eluted with sugar-containing buffer, made 4 M in urea. Finally, the radioactivity remaining on the column was measured. The figures represent the percentage, relative to the total radioactivity applied, of the radioactivity in the unadsorbed fraction, in the fraction eluted with sugar, in the fraction eluted upon addition of urea, and the amount remaining on the column after the final elution (residual).

When placenta membranes were crosslinked covalently with ¹²⁵I-labelled EGF-URO, either by the glutaraldehyde method or by the photoaffinity technique, electrophoretic analysis of the solubilised membrane protein in polyacrylamide slab gels revealed an identical autoradiographic pattern (Fig. 1); two major components (bands I and II) of apparent molecular weights 180,000 and 160,000 could be detected. The relative amounts of the two components present seemed to vary somewhat from one preparation to another, as indicated by differences in the intensity of the autoradiographic patterns. When crosslinking was carried out in the presence of an excess of unlabelled EGF-URO, the labelling of bands I and II was virtually abolished, whereas that of other barely detectable components remained unchanged.

Previous data^{15,21,23,24} suggested that the EGF-URO receptor is a glycoprotein. We observed that a variety of lectin-Agarose columns could adsorb selectively the major labelled components present in the crude solubilised membrane preparation (Table 1 and Fig. 1), enabling selective desorption with the appropriate lectin-specific sugar to take place. The wheat germ agglutinin (WGA)-agarose column proved to be the most effective, resulting in a 30-fold receptor purification using crude soluble preparations as starting material. Analysis of the specific eluate from the WGA column revealed the presence of a third minor radioactively-labelled component of apparent molecular weight 48,000.

previously observed with the antibody-agarose column was not detected. Similarly, when crude soluble membrane proteins were purified by WGA-agarose affinity chromatography, labelled with ¹²⁵I, and subsequently applied to an anti-EGF-URO antibody-agarose column and to pre-immune rabbit serum-agarose column, >95% of the radioactivity was unadsorbed. When the formic acid eluate was subjected to electrophoresis, bands in the region of I and II were not detected.

Because of the high specificity of the binding of EGF-URO to membrane-binding sites^{8–12,16,22} and because of the correlation of this binding with the biological activity of EGF-URO in certain cell systems^{8,10}, it is reasonable to assume that binding components detected in this and previous studies represent the cellular receptor involved in EGF-URO action. It is of particular importance that the two independent methods of radiolabelling the receptor yield identically labelled components, as indicated by electrophoretic analysis. It is perhaps surprising, as previously discussed²¹, that the glutaraldehyde-borohydride technique does not yield a wide spectrum of crosslinked components, but probably links ¹²⁵I-labelled human EGF-URO to its binding site in a relatively restricted membrane environment. Important is the fact that, using the conditions we describe, the photoaffinity label does not, following generation of the nitrene, alkylate a large number of protein constituents, as is observed in intact cells²². Because the two methods of radiolabelling the receptor are chemically quite dissimilar and

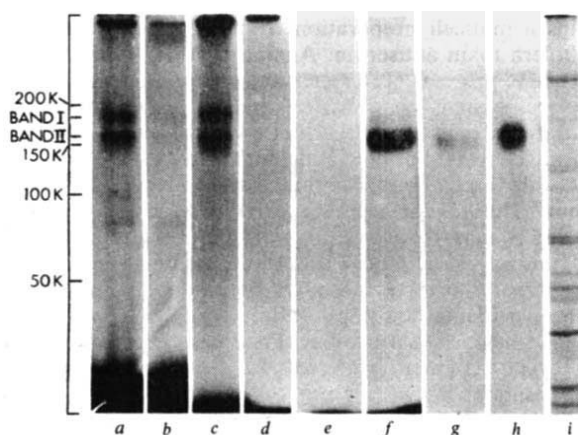


Fig. 1 Electrophoretic analysis and autoradiography of soluble protein from ^{125}I -EGF-URO affinity-labelled placenta membranes. Columns *a* and *b* represent the analysis of protein that was affinity-labelled either in the absence (*a*) or presence (*b*) of excess unlabelled mouse EGF-URO using the glutaraldehyde-borohydride method described previously²¹. Briefly, human placenta membranes (0.5 mg ml^{-1}), prepared by differential centrifugation, were equilibrated (40 min, 23°C) with ^{125}I -labelled human EGF-URO, washed with ice-cold buffer, resuspended, crosslinked with the addition of glutaraldehyde (4.2 mM ; 40 min, 4°C), reduced with sodium borohydride (16.7 mM , $\text{pH } 9.0$, 30 min, 4°C), solubilised (1% w/v Nonidet P-40, Particle Data Laboratories), clarified by centrifugation ($200,000\text{ g}$, 30 min) and the soluble supernatant analysed by polyacrylamide gel electrophoresis using 7.5% gels and the method of Laemmli²⁷. Labelled protein was detected by autoradiography of fixed, dried gels. Columns *c* and *d* represent the analysis of soluble protein from membranes that were first photoaffinity-labelled either in the absence (*c*) or presence (*d*) of excess unlabelled mouse EGF-URO. The photoactive ^{125}I -EGF-URO derivative was prepared by first iodinating mouse EGF-URO, using the Iodogen (Pierce) procedure as described previously²⁹, with $5\text{ }\mu\text{g}$ of mouse EGF-URO and 2 to 3 mCi carrier-free ^{125}I in $200\text{ }\mu\text{l}$ of 0.3 M phosphate buffer, $\text{pH } 7.6$. After the addition of ^{125}I (40 s), the reaction mixture was transferred to a foil-covered vial containing $800\text{ }\mu\text{l}$ 0.1 M phosphate buffer, $\text{pH } 7.6$. Aliquots were removed for measurement of the amount of radioactivity present and of the incorporation of ^{125}I into EGF-URO. The volume was made up to 2 ml with buffer and *N*-succinimidyl-6-(4'-azido-2'-nitrophenyl-amino) hexanoate (SANAH; Pierce) was added in the dark ($30\text{ }\mu\text{l}$ of 1 mg ml^{-1} in dimethylsulphoxide) to the stirred solution. The reaction (30 min , 24°C) was terminated by the addition of 0.5 ml of 50 mM unbuffered lysine HCl. The product was stored in the dark at 4°C and used without further purification. Photoaffinity-labelling was achieved by incubating the SANAH derivative of ^{125}I -labelled mouse EGF-URO (330 pmol) with membranes (40 ml of 1 mg membrane protein per ml in 0.1 M NaCl, 50 mM Tris HCl, $\text{pH } 7.6$) for 40 min at 23°C in the dark and then activating the azide group by illumination (two 200 W incandescent bulbs at 10 cm) at 4°C for 10 min . Labelled membranes were collected and washed twice by centrifugation ($48,000\text{ g}$ for 20 min), solubilised ($200,000\text{ g}$, 30 min) analysed by gel electrophoresis with visualisation of labelled protein by autoradiography. Columns *e* and *f* represent the analysis of soluble photoaffinity-labelled protein which was applied to a $300\text{ }\mu\text{l}$ wheat germ agglutinin (WGA)-Sephacrose 4B column (4 mg WGA ml^{-1} beads) prepared by the method of March *et al.*²⁸ and equilibrated with buffer (0.1% w/v Nonidet P-40, 0.2 M NaCl in 50 mM Tris HCl, $\text{pH } 7.6$). Column *e* represents the analysis of the unadsorbed fraction; column *f* represents the adsorbed material that was eluted from the WGA-Sephacrose column with 0.2 M *N*-acetylglucosamine (NAG) in buffer. Columns *g* and *h* show the analysis of protein that was first specifically eluted with NAG from WGA-Sephacrose and then applied to an anti-mouse EGF-URO antibody-Sephacrose 4B column. Column *g* represents the unadsorbed fraction; *h* shows material that adsorbed to the column and was eluted with 10% v/v formic acid. Column *i* shows the protein pattern of untreated placenta membranes stained with Coomassie Blue. Bands I and II represent the major affinity-labelled proteins; the left hand scale shows the calibration of the gel according to the molecular weight markers β -galactosidase (130K), phosphorylase A (94K), bovine serum albumin (68K) and pyruvate kinase (54K).

yet yield identical results, we conclude that the risks of misinterpretations inherent in either crosslinking procedure have been minimised.

The apparent molecular weights of the two principal constituents detected in the present study are in excellent accord with our own previous data²¹ and with the data of Das *et al.*²², who detected a photoaffinity-labelled substituent of apparent molecular weight of $195,000$ in mouse cells. We did not, however, detect, in the placenta membrane, the constituent of molecular weight $\sim 100,000$ which we observed in rat liver membranes using the glutaraldehyde-borohydride technique²¹. Detection of more than one receptor constituent cannot be readily explained. The most likely explanation, however, is that the two major constituents represent molecules that are closely related to one another either by a degradative (for example, receptor processing) or synthetic (precursor-product) process. These possibilities may explain the variation in the relative amounts of the two major constituents that we have observed from one membrane preparation to another. It is of interest in this regard that the affinity labelling of the β -adrenoreceptor also yields two principal constituents²⁵. No components analogous to the 'processed' receptor constituents detected by Das *et al.*²⁶ in an intact cell system seem to be detected by our techniques in the placenta membrane preparation.

The present work demonstrates directly the glycoprotein nature of the EGF-URO receptor. It also demonstrates a novel method for purifying receptors utilising anti-ligand antibodies and provides a scheme for the large scale isolation of receptors which, like the EGF-URO receptor, seem to lose their ability to recognise ligand after solubilisation and thus are not amenable to purification by hormone-agarose affinity chromatographic procedures.

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The molecular nature of heat-labile enterotoxin (LT) of *Escherichia coli*

ALTHOUGH *Escherichia coli* is part of the natural flora of the mammalian gut, certain strains cause a cholera-like disease in humans and young farm animals^{1,2}. These *E. coli* strains carry at least two plasmid-mediated determinants of virulence^{3,4}. One class of plasmids, called Ent, codes for the synthesis of one or more enterotoxins³. These toxins disrupt the fluid balance in the gut resulting in a net efflux of fluid and electrolytes from the gut epithelial cells¹. The heat-labile toxin (LT) and cholera toxin have been shown to be identical in their modes of action⁵ and also to be immunologically similar^{6,7}. Although cholera toxin has been purified and characterised⁸, it has proved difficult to purify LT and it is not yet fully characterised. Molecular weight estimates for LT range from 20,000 to over 100,000 (refs. 9–11). We report here investigations of the molecular nature of LT carried out by studying an LT gene isolated using recombinant DNA technology¹². Using *E. coli* minicells¹³, the proteins encoded by the LT gene were identified and the structure of LT was found to be similar to that of cholera toxin.

Recently we reported the isolation of an LT gene on a 5.8×10^6 -MW DNA fragment¹². Using *in vitro* deletion techniques, this DNA fragment has been reduced in size to MW 1.2×10^6 and has been linked to the R-plasmid pBR313 (W.S.D., D.M. Gill and S.F., in preparation). This new plasmid, EWD299, encodes for LT as measured by the Y-1 adrenal cell tissue culture assay¹⁴. Proteins specified by the chimaeric plasmid were identified using minicells as an *in vivo* protein synthesising system. Minicells do not contain chromosomal DNA but may contain plasmid DNA molecules. The plasmids are transcribed and the mRNA is translated in minicells.

Plasmid-encoded proteins from minicells were radioisotopically labelled by incubating purified minicells with ³⁵S-methionine or ¹⁴C-amino acid mix. The total minicell proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and the plasmid-specified proteins were identified by autoradiography. EWD299 was found to encode about 12 distinct proteins, (Fig. 1 track a). The LT gene-specified proteins were identified among these labelled proteins using an antiserum prepared against a crude preparation of LT and a *Staphylococcus aureus* strain containing protein A¹⁵. The protein A on the *S. aureus* binds antibodies without disrupting their active sites. Tertiary complexes of protein A, antibody and antigen can be formed and these aggregates can be separated from the unreacted antigens in the minicell lysate (see Fig. 1 for details). A 26,000-MW protein (Fig. 1, track e), was shown to bind nonspecifically to *S. aureus* cells and has been identified as β -lactamase¹⁶. Two other proteins of molecular weights 11,500 and 25,500 were found to be complexed in the presence of the anti-LT serum (Fig. 1 track d). These results may be interpreted in two ways. First, only one of the proteins is actually LT and the other protein was present in the extract used to raise the antiserum. On the other hand, LT may be a multimeric toxin composed of two distinct protein species.

Cholera toxin is a multimeric toxin composed of two structurally and immunologically distinct proteins⁸. Antisera can be prepared directed specifically against the adsorption protein, subunit B. This subunit binds the holotoxin to the target cell membrane, facilitating the activity of the A subunit, and has no toxic activity on its own. Antiserum prepared against subunit B was used in the *S. aureus* experiments and the results are shown in track b. Both the 11,500 and the 25,500-MW proteins were present, suggesting that LT is composed of at least two distinct proteins, one of which is immunologically related to the B subunit of cholera toxin. These data indicate that LT is present as a multimeric toxin intracellularly, as two proteins were isolated with an antiserum directed against a single protein. Track c

contains a minicell preparation from an experiment using a holocholera toxin antiserum. Again, both the 11,500 and the 25,500-MW proteins are present.

Cholera toxin is composed of three polypeptides. The B subunit migrates in SDS-PAGE at an apparent MW of 11,000 (data not shown). Subunit A is composed of a 24,000-MW polypeptide linked by a disulphide bond to a 5,500-MW polypeptide¹⁷. The subunit structure we have found for LT is similar to that of cholera toxin but there are notable differences. The small subunit of LT, the 11,500-MW protein, is about 500 daltons larger than the B subunit of cholera toxin. Furthermore, there is no evidence of disulphide linkage in the 25,500-MW LT subunit. It has been shown that subunit A of cholera toxin has adenyl cyclase stimulating activity and a similar activity in crude LT preparations has been seen²⁰. We also have enzymatic and genetic evidence that demonstrates an adenyl cyclase stimulating activity for the 25,500-MW protein (Dallas, Gill and Falkow, in preparation).

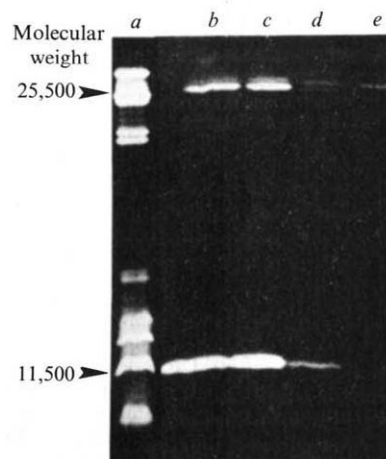


Fig. 1 Identification of the LT proteins. Minicells containing EWD299 were isolated and plasmid-encoded proteins were labelled by incubating the minicells with ³⁵S-methionine¹⁶. A minicell cytoplasmic fraction was prepared as follows. Labelled minicells were frozen and resuspended in phosphate buffered saline, 1% Triton X-100, 5 mM EDTA (PBSTE). Lysozyme was added to $100 \mu\text{g ml}^{-1}$ and the preparation was sonicated. The lysate was cleared at $35,000g$ for 30 minutes and the supernatant was used for the antisera experiments. An aliquot of antiserum was added to the supernatant and the solution was incubated at room temperature for 30 min. Formalised *S. aureus*¹⁵ was added and the mixture was incubated for an additional 30 min. *S. aureus*-antibody-antigen complexes were isolated by pelleting the solution through 1 M sucrose in PBTSE (1,000g). The pellet was boiled for 3 min in final sample buffer (10% glycerol, 5% β -mercaptoethanol, 3% SDS and 0.0625 M Tris, pH 6.8). Aliquots were electrophoresed on 10–15% gradient SDS-polyacrylamide gels¹⁹. The gels were dried onto filter paper and autoradiographed for 3–14 d. Track a, total minicell lysate; track b, anti-cholera B subunit serum; track c, anti-cholera toxin serum; track d, anti-LT serum; track e, no anti-serum added.

The primary structure of cholera toxin subunit B is established¹⁸. It should be possible to determine the primary structure of the 11,500-MW LT protein by nucleotide sequencing and to compare the amino acid sequences of these two proteins. Analysis of the primary structure of the two large toxin proteins may give further information concerning the evolution of these two similar enterotoxins.

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Escherichia coli lac operator mRNA affects translation of β -galactosidase mRNA

THE primary function of the start of a message (mRNA for a specific polypeptide) is to ensure the correct initiation of translation. Little is known about the factors determining the frequency of translation or indeed if its variability is important in the total expression of genes. Recently, an analysis was introduced that allows calculation of *in vivo* frequencies; it was shown that the *in vivo* initiation rates of the first and last messages of the *lac* mRNA differ by fivefold¹. Different initiation frequencies could be effected, in part at least, by a sequence of three or more nucleotides about 8 to 15 residues upstream from the initiating AUG codon that is complementary to a purine-rich sequence at the end of the 16S ribosomal RNA^{2,3}. Four such complementary nucleotides are residues 8–11 before the AUG start of the β -galactosidase (EC 3.2.1.23, β Gase) message. Mutants for this sequence are not available, but there is a whole series for a more distant region—22 to 34 nucleotides before the AUG: the *lac* O^c mutants. Here we show that certain of these mutations affect the rate of translation as well as the stability of this message.

Lac O^c mutants are defective for binding repressor protein to the *lac* operator. The more than 800 O^c mutants of Smith and

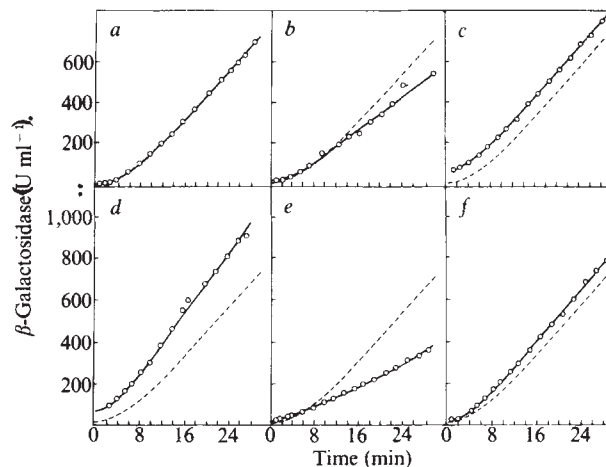


Fig. 2 The induction of β Gase in various *E. coli* HfrC, *proC*, *metB*, *strS* *lac* O^c mutants and O⁺ parent strain described by Sadler and Smith⁴. Bacteria were grown at 37 °C in M9 inorganic salts²⁶ with methionine (to 50 μ g ml⁻¹), proline (to 50 μ g ml⁻¹), casein hydrolysate (to 0.4%) plus 0.2% glycerol and were induced at zero time by the addition of isopropyl- β -D-thiogalactoside (IPTG) (to 0.5 mM) after addition of cyclic AMP (to 2 mM) at -2 min to minimise catabolite repression. Samples were removed at the indicated times and brought to 0 °C, and metabolism further inhibited by addition of chloramphenicol (to 50 μ g ml⁻¹) plus sodium azide (to 10 mM). The rate of β Gase synthesis was calculated after 15 min of induction when it had reached its maximum. At this time there were 2.6×10^8 viable bacteria per ml with 32 μ g RNA per ml. *a*, Parent strain O⁺; *b*, strain IIIa; *c*, strain IIB; *d*, strain IIBb; *e*, strain IIIb; *f*, strain Vb. The synthesis of β Gase in the parent strain (*a*) is reproduced for comparison as a dashed line in each panel.

Sadler⁴ could be grouped into seven classes defined by *P* values (ratio of basal to induced β Gase levels). So far, representatives of each class have been identified as single-base change mutants⁵ which map in the initial sequences of the *lac* mRNA⁶ (Fig. 1). This primary effect on the repressor-operator binding does not explain other changes in β Gase expression. The amount of fully induced β Gase (zero repression) differs between strains, with some accumulating more enzyme, and some less, than does the O⁺ parent^{4,7}. Thus, it seemed possible that the translation initiation frequency was affected by these mutations.

Rates of β Gase synthesis during full induction are required to estimate translation-initiation frequencies. Of six representative strains shown in Fig. 2, IIB and Vb are similar to the parent, IIB gives stronger induction, and IIIa and IIIb give weaker. The ratios of induction rates seen here between strains are similar to the ratios of fully induced enzyme levels observed earlier^{4,7}. The turnover number for β Gase is $\sim 400,000$ U mg⁻¹ (refs 1, 8) and its tetrameric molecular weight is 465,000 (ref. 9). With these values and the viable cell count determined at the time of induction measurement, the synthesis of β Gase monomers per s

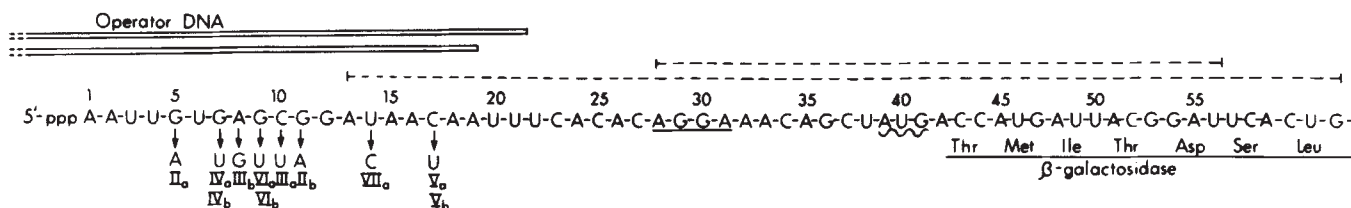


Fig. 1 The nucleotide sequence of the start of the *lac* mRNA as determined by Maizels⁶. The base substitutions expected in the mRNA from the changes in DNA sequence of the various O^c strains, determined by Gilbert *et al.*⁵ are shown. The wavy line designates the initiation codon for the start of the β Gase polypeptide and the underlined AGGA could be a potential recognition site for UCCU at positions 3–6 from the 3' end of the 16S rRNA. The sequences derived from the right side of the operator DNA, as defined by protection from repressor binding²⁴, as well as the shorter length defined from the minimal stretch that can form stable repressor complex²⁵, are shown by the horizontal bars. The dashed lines show the residues protected from RNase degradation by a ribosome initiation complex. The longer segment is the maximum length protected against RNase T1 and the shorter is the minimum length protected against RNase A¹³.

cell is calculated in Table 1, column 2. The parent HfrC strain produces 19 monomers per s cell, strain IIa 25, and at the other extreme, IIIb only nine.

Three parameters of gene expression combine to give these final rates of enzyme synthesis at any instant. (1) The number of message molecules producing finished polypeptides, (2) the rate of translation initiation, and (3) the rate of ribosome movement. The first parameter is the most difficult to measure. It is derived from the total *z* mRNA mass which is given most accurately by dividing its rate of synthesis (*S*) by its decay constant (*k*) (refs 1, 10) and then converting mass to numbers of completed molecules¹.

The rate of *z* message synthesis is derived from the fraction of 30 s pulse-labelled RNA that is *z*-specific in these fully induced cells (about 1.5% after correction¹ for hybridisation efficiency). As these strains double every 50 min, they contain 1.7 copies of the *lac* operon per cell¹; this gives about 2.5 s between transcription initiations and 100 nucleotides between RNA polymerases (column 3). These mutations do not affect the rate of transcription initiation. None of the strains show functional or mass decay slower than that of the parent ($t_{1/2} = 1.5$ min) (Fig. 3), but decay in IIa, IIIa, Va, Vb and VIa is significantly faster ($t_{1/2} < 1.2$ min) (column 4).

After subtracting the mass of nascent *z* mRNA, the number of completed molecules can be calculated (column 5, and Table 1 legend). Combined with the rate of ribosome movement (about 40 nucleotides per s per ribosome from the 80 s induction lag^{11,12}) the translation initiation rate (column 6) and resultant density of ribosomes can be calculated. The loading frequency of the parent averages 4.9 s between initiations to give 176 nucleotides between ribosomes. Six O^c strains differ: IIa, Va, Vb and VIa average only 3 s between translation initiations (110 nucleotides between ribosomes), whereas IIIb and IVb initiate

significantly more slowly (8 s between initiations for strain IIIb to give 288 nucleotides between ribosomes.) There is a threefold range of initiation rates between these strains.

Mutations in the fastest-loading strains are not clustered; IIa and Va/Vb are 12 residues apart, and in fact, define the outer boundaries of the set. Conversely, the two mutations that give slower-loading are adjacent. All of these mutations lie outside the RNA region protected from RNase A digestion by the initiating ribosome¹³ (Fig. 1). The IIa mutation is located about the diameter of a ribosome from the AUG start (assuming 4 Å per residue).

A possible secondary structure of the operator RNA can be considered (Fig. 4). Its free energy can be calculated from studies with synthetic polyribotides¹⁴⁻¹⁷. The favourable free energy from base pair stacking is about -8.6 kcal mol⁻¹ at 25 °C, and the unfavourable energy from the constraints in closing the loop¹⁴ would be about $+7$ kcal mol⁻¹ (for a 9-base loop closed by AU) to give a net -1.6 kcal mol⁻¹ at 25 °C. This structure should 'melt' at >25 °C; the T_m can be estimated from the enthalpy and entropy values that were also measured for the model polymers. Using values from two laboratories^{14,15} yields a T_m of 45 °C or 58 °C. Note that the T_m of a monomeric duplex (with hairpin loop of this size) is much higher than is the T_m of the same duplex formed from two separate strands (see Fig. 4 in ref. 14). While the melting profile may be quite broad, it seems likely that most of the molecules are in this configuration at 37 °C.

What is striking is that the fast-loading strains IIa, Va, Vb give very similar decay and loading values and share the common feature of disrupting the single GC bond in the stem (Fig. 4) to generate a stem whose energy is now limited to the lower four AU pairs (-3.6 kcal) and has considerably less energy than the $+8$ kcal in the 11-base loop. If the secondary structure were a hindrance to ribosome loading, then such base changes would

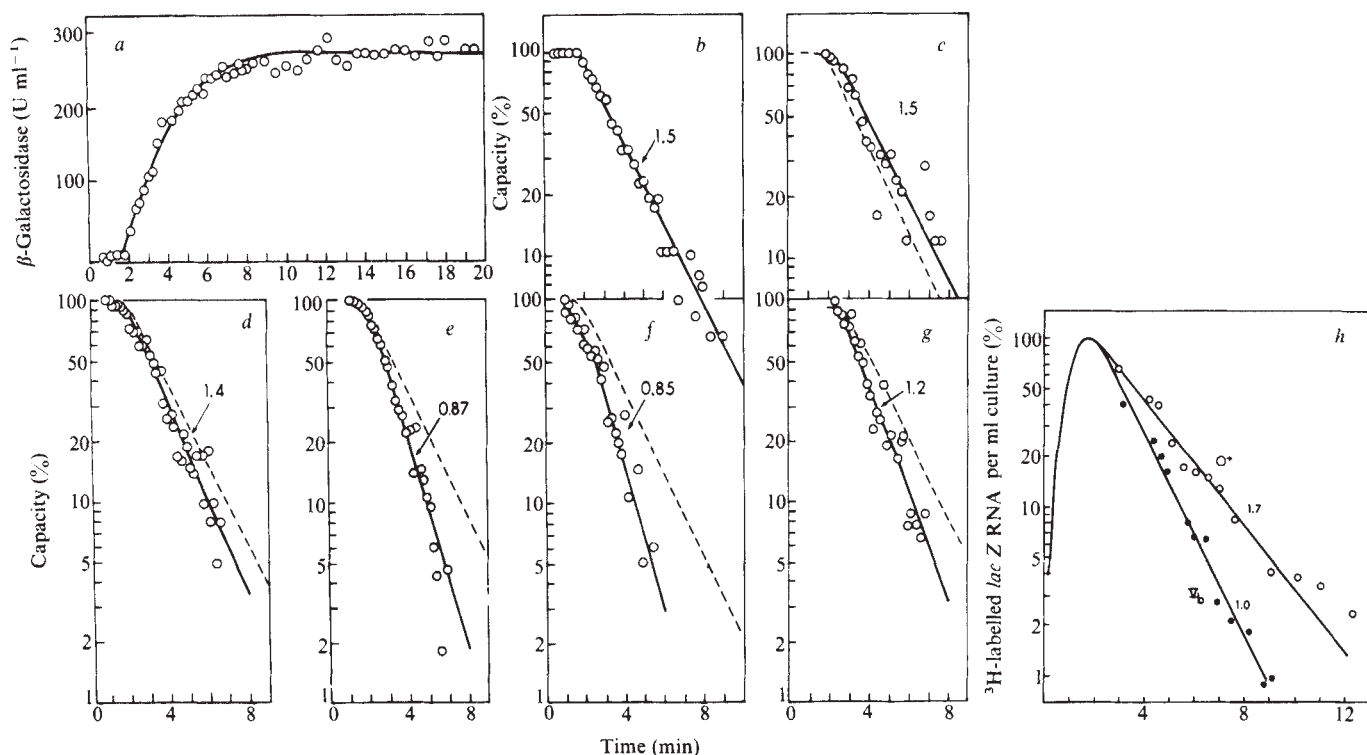


Fig. 3 Functional and mass decay of β Gase message in various *E. coli* *lac* O^c mutants and the O⁺ parent. Cultures were induced at zero time and sampled as described in Fig. 2 except that rifampicin (to 300 μ g ml⁻¹) was added at +30 s to inhibit further transcription initiations. *a*, The burst of β Gase from parent strain O⁺; *b*, the loss of capacity to synthesise enzyme²⁷ from the data in (*a*). The remaining panels only show this latter variable for each strain induced and sampled in the same way. *c*, Strain IIb; *d*, strain IIIb; *e*, strain Vb; *f*, strain IIIa; *g*, strain IIa. The half lives (min) are shown and the curve of the parent strain (*b*) is reproduced for comparison in each panel. Panel (*h*) shows the mass decay of β Gase message in strain Vb and parent O⁺ strain; [5,6-³H]uridine (to 33 μ l ml⁻¹ and 2 nmol ml⁻¹) was added at -30 s and rifampicin as above. RNA extracted from each sample was reacted with denatured λ plac 5 DNA to form hybrid and the hybrid fraction times the amount of ³H-RNA per ml gives the relative amount of *lac z* message shown²⁸. The half lives (in min) are shown.

Table 1 Expression of β -galactosidase gene in operator constitutive mutants of *E. coli*

Strain	1	2	3	4	5	6
		β Gase synthesis monomers per cell per s*	Nucleotides per RNA polymerase	mRNA half-life(s) (ln 2/k)	Completed mRNA molecules per cell†	Time between translation initiations (s)‡
O ⁺		19	97	90	90	4.9
Ila		25	113	69	68	2.6
Iib		19	97	90	90	4.8
IIla		12	90	54	63	5.1
IIlb		9	117	84	70	8.0
IVa		24	90	84	92	3.9
IVb		12	101	75	85	6.8
Va		23	95	69	81	3.3
Vb		19	84	57	67	3.4
VIa		20	97	63	70	3.2
VIb		15	105	78	86	5.3
VIIa		16	115	72	71	4.2

Exponentially growing bacteria were induced by addition of cyclic AMP (to 2 mM) and IPTG (to 0.5 mM) and rates of enzyme and message synthesis measured between 12 and 20 min after IPTG addition. At 15 min there were 2.6×10^8 cells per ml with 32 μ g RNA per ml. Divide amounts per cell by 1.66 to give amounts per chromosome. All values refer to the β Gase gene, mRNA or enzyme.

* There are four monomers per β Gase to give 4.8 molecules of β Gase per s per cell in strain O⁺.

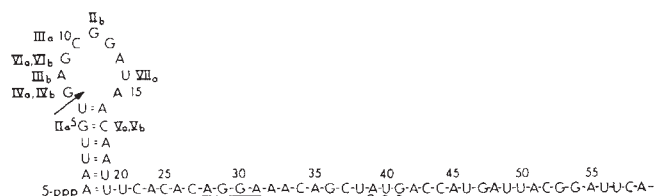
† The total mass of *lac z* mRNA/cell is made of: (a) completed *z* molecules that are not decaying, (b) completed *z* molecules in various stages of decay, and (c) incomplete or nascent molecules (some are also decaying), still on DNA and thus not releasing finished polypeptides at that instant. The mass of the nascent class can be estimated from *S* and subtraction from the total mass gives the mass of functioning molecules. The distribution between decaying and non-decaying species is derived from the 5' to 3' nature of the decay process¹ and leads to the actual numbers of functioning molecules per cell (column 5) (see ref. 1). The total *lac z* mRNA per cell varies from 1.5×10^{-10} μ g (three strains including O⁺) to 1.0×10^{-10} μ g (four strains). There are 52 nascent molecules per O⁺ cell, 50 complete and 40 completed but decaying. The number of completed molecules that are not decaying is the most variable with the faster-decaying strains (IIa, IIIa, Va, Vb, VIa and VIIa) containing only about 28 molecules per cell.

‡ All experiments were performed on three different cultures of each strain. The experimental values (*S*, *k*, and induction rate) represent averages with maximum deviations of $\pm 10\%$ each. If the experimental values for a strain were all in error by 10% 'in the same direction', the final computer-derived initiation frequency (column 6) would be in error 30% in that direction—insufficient to account for the 300% range of values.

decrease the probability of its existence and thus increase the frequency of successful collisions.

The slower initiation rates of strains IIIb and IVb might also reflect the presence of the secondary structure of Fig. 4. First, note that IVb would generate two more base pairs and a five-membered loop to increase the structural stability to $\Delta G = -5.1$ kcal mol⁻¹ at 25 °C from -1.6 kcal mol⁻¹. However, IIb would not affect the structural stability and suggests another possible explanation. Independent studies in this laboratory have shown an involvement of RNase III activity in translation initiation of β Gase. β Gase mRNA from strain AB301-105 (ref. 18) is much less competent to form *in vitro* initiation complexes than is β Gase mRNA from the *mc*⁺ parent⁹. The RNase III recognition sequence has been defined in detail in four of six T7 RNA sites^{20,21}, and the four are similar: cleavage between U and G and release of terminal fragments UUAU_{OH} and pGAU. Cleavage of *lac* RNA between the residues U and G at positions 6 and 7 would liberate UUGU_{OH} plus pGAG. As indicated above, opening the loop would lower the *T_m* of the duplex. If this is a site for RNase III cleavage, then IIIb and possibly IVb, might load ribosomes more slowly because the enzyme could no longer disrupt the structure. The hypothesis is being tested by *in vitro* experiments.

Strains Vlb, IIIa, Iib and VIIa give the parental O⁺ initiation frequency, and they all map in the upper part of the loop structure and should not alter it. Thus, 9 of the 11 characterised *lac* O^c mutants give expression that is consistent with the structure shown in Fig. 4. Strains VIa and IVa give inconsistent results. It is possible that they were assigned incorrectly or contain secondary mutations in either *lac* DNA or the genetic

**Fig. 4** A possible configuration of the *lac* mRNA⁶. The AGGA and AUG are designated as in Fig. 1. The arrow indicates a putative RNase III target.

background. In strains IVa and VIa expression of the distal *lac* galactoside acetyltransferase (GATase) message and β Gase message change in parallel, suggesting some nonspecific effect, perhaps on all messages. In contrast, for example, the extremely fast β Gase message decay in strain Vb is not paralleled by faster than normal (O⁺) decay of GATase message (data not shown).

Thus, the major observation presented here is that the RNA region more than 21 residues before an AUG start is important for translation initiation as well as message stability. These two processes show no obvious correlation here (Table 1); however, other studies suggest a causal relationship between decay and loading frequency^{1,22}, perhaps obscured in these base change mutants because the vulnerability of the specific decay target must also be a function of the primary base sequence itself. A role of RNA secondary structure in initiation has been suggested, not only in the well known RNA phage translation, but in recent speculation concerning internal initiation sites of the *lac i* mRNA²³ which have potentially base-paired regions proximal to them. However, this is the first report to relate mutations in a potential secondary structure to measured changes (threefold range) in initiation frequencies.

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Double-stranded DNA stereoselectively binds benzo(a)pyrene diol epoxides

BEFORE it can exert its mutagenic and carcinogenic activities^{1,2} benzo(a)pyrene (BP) requires metabolic activation and it is widely considered³⁻⁵ that the initiating event in these processes is covalent binding to DNA. The predominant form of the activated hydrocarbon is a diol epoxide⁶⁻¹¹, of which four possible isomers are (+) and (-) enantiomers of 7 β ,8 α -dihydroxy-9 α -10 α -epoxy-7,8,9,10-tetrahydro-BP (*anti*-BP diol epoxide) and (+) and (-) enantiomers of 7 β ,8 α -dihydroxy-9 β ,10 β -epoxy-7,8,9,10-tetrahydro-BP (*syn*-BP diol epoxide). Enzymatic formation of the (+) *anti* and (+) *syn* configurations have been reported elsewhere^{12,13}. We have shown that the racemic ($\pm$) *anti*-BP diol epoxide forms two stable covalent adducts each with the exocyclic amino groups of deoxyguanosine, deoxyadenosine and probably deoxycytidine¹⁴. We report here that we have now resolved the enantiomers of ($\pm$) *anti*-BP diol epoxide and reacted the optically pure hydrocarbons with DNA. Each enantiomer reacts with the exocyclic amino group of deoxyguanosine to form a pair of diastereomers in a ratio which depends on the secondary structure of the nucleic acid. The (+) enantiomer reacts preferentially or asymmetrically with deoxyguanosine in double stranded DNA but the two enantiomers react equally with the same position in single-stranded DNA. The basis for the stereoselective binding is unknown but may involve formation of sterically orientated physical complexes before covalent attachment of the hydrocarbon to DNA. The mode of binding in the deoxyadenosine sites, on the other hand, must be different, as the (+) and (-) enantiomers react equally in either single- or double-stranded DNA.

The ($\pm$)BP diol epoxide was synthesised as described elsewhere^{15,16}. ³H-($\pm$)*anti*-BP diol epoxide was synthesised by an adaptation of the method used by McCaustland *et al.*¹⁷. The (+) and (-) enantiomers of *trans*-7,8-dihydroxy-7,8-dihydro-BP were resolved by published methods¹⁸ and converted to (-) and (+) *anti*-BP diol epoxides, respectively. Either racemic ($\pm$) *anti*-BP diol epoxide or the optically pure (>96%) (+) or (-) enantiomers were reacted with calf thymus or Φ X174 DNA. After removal of unreacted hydrocarbon the DNA was enzymatically hydrolysed to hydrocarbon-deoxynucleosides and the stable covalent adducts isolated by Sephadex LH-20 column chromatography. The adducts were then separated by reverse-phase high-pressure liquid chromatography (HPLC). Of the stable covalent adducts formed, 95% were recovered as determined by radioactivity measurements. Figure 1a shows the HPLC profile obtained by reacting racemic ($\pm$) *anti*-BP diol epoxide with native calf thymus DNA after hydrolysis and Sephadex LH-20 column chromatography.

We have previously identified adducts numbered 1-5, 7 and 8 (ref. 14). Peak number 1 was a tetraol hydrolysis product of the *anti*-BP diol epoxide, whereas adducts 2 and 4 represented deoxycytidine-derived products. The contribution of peak 4 to the total adduct formation was variable as it co-chromatographed with one of the tetraol hydrolysis products of the diol epoxide. Adducts 3 and 5 were reaction products with deoxyguanosine, and numbers 7, 8 and 9 were deoxyadenosine products. With native calf thymus DNA the deoxyguanosine adducts represented 90% of those formed. However, the distribution of adducts obtained depended on the reaction conditions used and was primarily a function of DNA secondary structure, that is, the more denatured the polymer the higher the proportion of adenine adducts obtained.

We have previously reported¹⁴ that the pair of deoxyguanosine adducts (peaks 3 and 5) obtained from reaction of racemic ($\pm$) *anti*-BP diol epoxide with native calf thymus DNA were probably diastereomers, as their high resolution mass spectra were identical and their circular dichroism spectra were

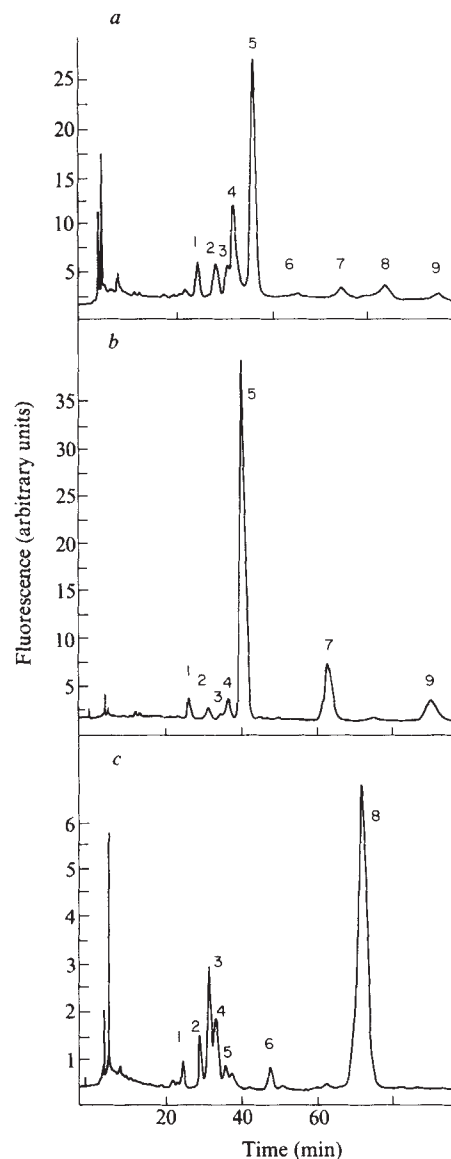


Fig. 1 Tritium-labelled racemic ($\pm$) *anti*-BP diol epoxide or the resolved enantiomers were incubated with 1 mg deproteinised native calf thymus DNA at an initial concentration of 1 hydrocarbon per 250 nucleotides in 0.01 M sodium phosphate, pH 7.5, for 1 h at 37°C. Unbound hydrocarbon was removed by ethyl acetate extraction and repeated isopropanol precipitation of the DNA until zero time blanks contained <3% of the tritium of incubated samples. Routinely, incubated samples resulted in a modified DNA of 1 hydrocarbon per 8,000 base pairs. The diol epoxide-treated DNA was converted to hydrocarbon-deoxynucleosides and deoxynucleosides by treatment with DNase II, spleen phosphodiesterase and alkaline phosphatase. The hydrolysed sample was applied to a Sephadex LH-20 column and the free deoxynucleosides and enzymes eluted with water. The hydrocarbon-deoxynucleosides were quantitatively recovered with methanol and analysed by HPLC¹⁴. The elution profiles represent DNA reacted with: a, racemic ($\pm$) *anti*-BP diol epoxide; b, (+) *anti*-BP diol epoxide; c, (-) *anti*-BP diol epoxide. The identity of adducts 1-9 are discussed in the text.

of similar shape but inverted. We have now demonstrated this result for both the deoxyguanosine and deoxyadenosine products by resolving the enantiomeric diol epoxides and carrying out adduct formation individually with DNA. The results of these reactions are shown in Fig. 1b, c. These profiles indicate that the (+) enantiomer reacted to form deoxyguanosine adduct 5 and deoxyadenosine adducts 7 and 9, whereas the (-) enantiomer resulted in deoxyguanosine adducts 3 and 6 and deoxyadenosine adduct 8. Peaks 6 and 9, although not previously

identified, correspond to deoxyguanosine and deoxyadenosine adducts, respectively, as indicated by field desorption mass spectrometry¹⁹.

Reaction of nucleophiles with *anti*-BP diol epoxide has been shown to occur exclusively at the benzylic C-10 position, with a preference for *trans* addition²⁰⁻²². It is possible, however, that DNA secondary structure could influence the stereochemistry of the epoxide ring opening to give *cis* addition products as well. As the D-deoxyribose moiety of each nucleoside unit within the DNA polymer contains an asymmetrical centre at C-1, the reaction of a racemic mixture of ($\pm$)BP diol epoxide would be expected to yield a maximum of four diastereomeric products per alkylation site ((+) diol epoxide per D-deoxyribose, each with *cis* or *trans* addition at C-10 of the hydrocarbon). Reaction of the metabolically produced (+) *anti*- and (+) *syn*-BP diol epoxide^{12,13} with the exocyclic amino groups of guanine, adenine and cytosine in DNA would result in 12 possible diastereomeric covalent adducts. Recent indirect evidence²³ suggests that the (-) *anti* and (-) *syn* diol epoxides may also be made *in vivo*, and if this is the case then there would be 24 possible products by reaction of the four hydrocarbons with the three exocyclic amino groups in DNA. We obtained two resolvable deoxyadenosine diastereomers by reaction of the (+) *anti*-BP diol epoxide with DNA (adducts 7 and 9), which were probably the result of *cis* and *trans* addition of the adenine exocyclic amino group. Reaction of the (-) diol epoxide yielded peak 8 which by recycle chromatography consisted of two partially resolvable components. Each had virtually identical mass spectra¹⁹, indicating that they were deoxyadenosine exocyclic amino adducts. Thus, the (-) enantiomer reacted with deoxyadenosine to give two adducts, in agreement with the above predictions.

Three hydrocarbon-deoxyguanosine adducts were observed in the reaction of racemic ($\pm$) *anti*-BP diol epoxide with DNA—peaks 3, 5 and 6. Peaks 3 and 5 correspond to the major and,

presumably, *trans* addition products of (-) and (+) diol epoxide with N²-deoxyguanosine, respectively. Peak 6, derived from the (-) enantiomer, was a relatively minor component and may correspond to either an exocyclic amino *cis* addition product or to a different guanine adduct with alkylation at some other site. For deoxycytidine only two adducts (peaks 2 and 4) were observed. The relative contributions of (+) and (-) *anti*-BP diol epoxide to these two peaks was difficult to assess, as peak 4 also contained varying amounts of a tetraol hydrolysis product.

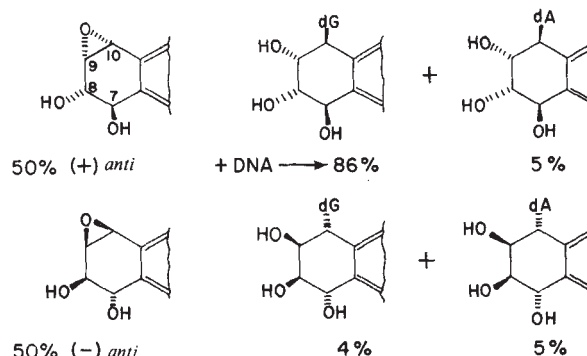


Fig. 3 The structure and per cent distribution of the principal guanine and adenine *trans*-addition products obtained by reacting racemic ($\pm$) *anti*-BP diol epoxide with native calf thymus DNA. The distribution also includes any *cis*-addition products (see text).

Figure 3 shows the structures of the *trans*-addition adducts and their per cent distribution obtained by reaction of racemic ($\pm$) *anti*-BP diol epoxide and native calf thymus DNA. (These percentages also represent any *cis*-addition products.) With the identification of adduct 9, we found that reaction of racemic ($\pm$) *anti* diol epoxide with DNA resulted in nearly equal distribution of deoxyadenosine diastereomer products, that is, adducts 7 and 9 from the (+) enantiomer of the hydrocarbon were about equal to adduct(s) 8 from the (-) enantiomer. By contrast, deoxyguanosine in DNA reacted stereoselectively with racemic *anti*-BP diol epoxide, that is, the ratio of adduct 5 derived from the (+) hydrocarbon to that of adduct 3 from the (-) enantiomer was 20:1. The same ratio of diastereomers was obtained by reaction of the optically resolved hydrocarbons and native calf thymus DNA (Fig. 1b, c). We initially postulated that the stereoselective binding could be a result of the hydrocarbons interacting asymmetrically with the secondary structure of DNA¹⁴. This postulate was tested by reacting racemic ($\pm$) *anti*-BP diol epoxide with double (Fig. 2a) and single (Fig. 2b) stranded Φ X174 DNA. The same distribution of stereoselective guanine adducts was obtained with native calf thymus DNA (Fig. 1a) and double-stranded Φ X174 DNA (Fig. 2a). Reaction of racemic ($\pm$) *anti*-BP diol epoxide with single-stranded Φ X174, however, resulted in nearly equal distribution of hydrocarbon-deoxyguanosine diastereomers (peaks 3 and 5). In addition, reaction with the single-stranded polydeoxynucleotide resulted in a significantly increased proportion of deoxyadenosine adducts. These same results were obtained with native and heat- or base-denatured calf thymus DNA. The ratio of deoxyguanosine diastereomers in the single-stranded calf thymus DNAs was approximately 1:1, and the proportion of deoxyadenosine adducts was 40% compared to 10% in the double-stranded polydeoxynucleotide.

These results demonstrated that the asymmetrical binding of the two enantiomeric *anti*-BP diol epoxides to the exocyclic amino group of guanine in double-stranded DNA was dependent on the secondary structure of the polymer. One possible mechanism for the ability of DNA secondary structure to distinguish two enantiomeric molecules is by stereoselective physical interactions (possibly intercalation) before covalent binding. Intercalation or some other physical interaction may readily

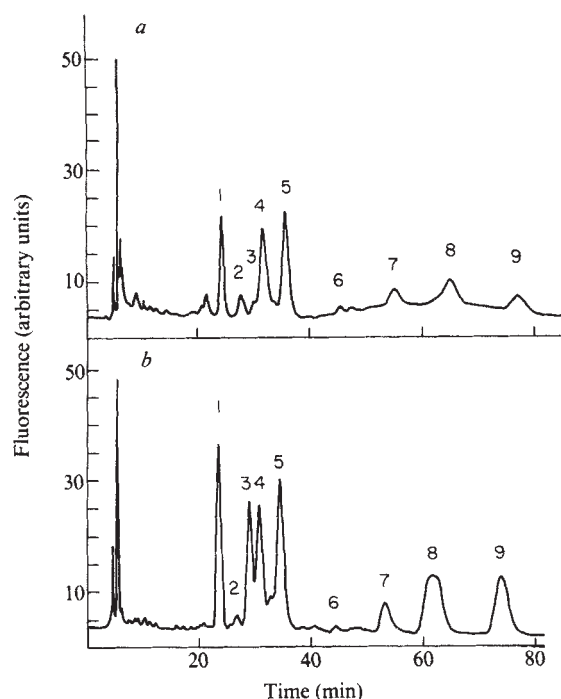


Fig. 2 Racemic ($\pm$) *anti*-BP diol epoxide was reacted with: a, double-stranded (replicative) Φ X174 DNA; b, single-stranded (viral) Φ X174 DNA. Adducts were formed and isolated as described in Fig. 1. The ratio of deoxyguanosine diastereomers derived from the (+) and (-) hydrocarbon is 20:1 for double-stranded DNA (the ratio of peaks 5 to 3, panel a), and 20:17 for the single-stranded polymer (the ratio of peaks 5 to 3, panel b). See text for discussion.

occur with the (+) enantiomer but not with the mirror image hydrocarbon. Alternatively, the noncovalent interactions could 'fix' the hydrocarbon so that only one configuration is in a favourable position to react with the exocyclic amino group deoxyguanosine. The following observations suggest that intercalation or some physical association between the BP diol epoxide and DNA take place before covalent binding: (1) as with intercalation of BP²⁴ or actinomycin²⁵ into DNA, binding of the diol epoxide occurs with a preference for guanine sites, and (2) reaction with denatured DNA 'randomises' adduct formation (loss of stereoselectivity and more adenine binding). We propose such an intercalation model to account for the stereoselective interactions between BP diol epoxide and DNA. The lack of any stereoselectivity in the adenine covalent binding sites with either double- or single-stranded DNA suggests that the reaction mechanism may be different from that of guanine binding. In the case of adenine the hydrocarbon may react without any physical interactions modulating the ratio of diastereomers obtained.

A correlation between DNA binding and carcinogenic or mutagenic potency has been reported for several polycyclic aromatic hydrocarbons³⁻⁵. Wood *et al.*²⁶ have determined the difference in the mutagenic activities of the (+) and (-) *anti*-BP diol epoxides using a mammalian tissue culture cell line and two bacterial tester strains. The difference in mutagenic activity between the (+) and (-) *anti* diol epoxides in the mammalian cell line was of the same order as we found for the difference in the level of covalent binding of the two isomers to the N²-guanine site in native DNA. Therefore, the extent to which these activated hydrocarbons react chemically with DNA *in vitro* correlates with their biological activities. These results suggest that, in addition to metabolic factors, chemical and physical interactions with DNA may be important in evaluating the biological potency of carcinogenic and aromatic hydrocarbons.

The regulation of gene expression usually involves protein-nucleic acid interactions²⁷. As we have demonstrated highly selective binding between small organic molecules and DNA, the possibility exists that this type of interaction may also have a regulatory role in the normal functioning cell.

We thank Dr A. Burlingame for the high resolution mass spectra. This research was supported by the Biomedical and Environmental Research Division of the US Department of Energy.

Note added in proof: Of the four isomers, (+) and (-) *anti*- and (+) and (-) *syn*-BP diol epoxides, the (+) *anti*-configuration is the most carcinogenic in newborn mice²⁸. Therefore, the extent of non-enzymatic, covalent binding of this enantiomer to double stranded DNA correlates not only with mutagenic activity²⁶ but also with tumorigenic potency.

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Hydrolysis of protein in vacuoles isolated from higher plant tissue

THE vacuoles of higher plant cells are known to be sites of deposition of salts and metabolites. A role as the lytic compartment has also been proposed¹, and Matile has argued that the vacuole participates in the metabolism of higher plant cells in the same way that lysosomes do in other organisms. Recently, isolation methods have been developed which allow a direct analysis of vacuolar constituents and we have shown previously that the vacuoles isolated from the endosperm of young castor bean seedlings contain at least 25% of the cellular protein, 62% of the sucrose and various hydrolytic enzymes (acid protease, carboxypeptidase, phosphodiesterase, RNAase, phytase and β -glucosidase) in amounts indicating a primarily (possibly exclusive) vacuolar localisation². In this tissue it is known that when the dry seed imbibes water the outer, water-soluble matrix of the protein bodies is dissolved and small vacuoles containing the protein crystalloid are formed. These coalesce early in germination to form the large central vacuole, and the remnants of the protein bodies disappear within a few days as growth proceeds³. We have examined vacuoles isolated from the endosperm of 4-d-old seedlings, when it is known that a net loss of protein is occurring. Here we compare the protein composition of the vacuoles with that of the original protein bodies, and show that hydrolysis of the endogenous protein continues in the isolated vacuoles which are free from other cellular components.

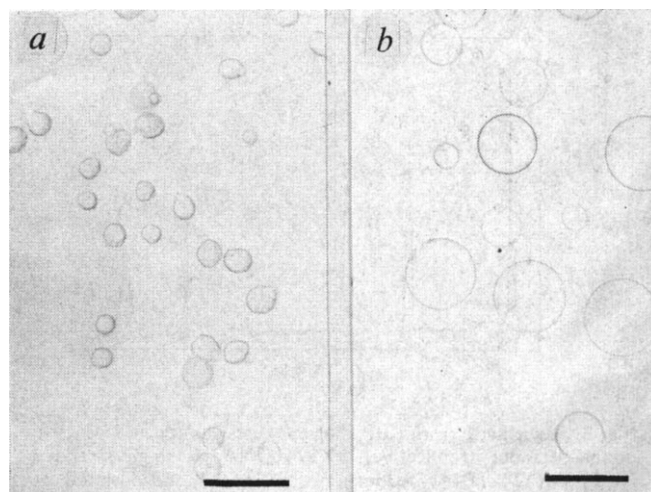


Fig. 1 Photomicrographs of *a*, protein bodies prepared from dry castor beans, and, *b*, vacuoles prepared from endosperm of 4-d-old seedlings and purified by passage into 40% sucrose containing 0.1 mM EDTA². Bars, 50 μ m.

Table 1 Protein content of protein bodies and vacuoles

	Protein bodies (mg per 10 ⁶)	Vacuoles (mg per 10 ⁶)
Total protein	0.71	1.92
Water soluble	0.14 (19.7)	0.90 (46.6)
Water insoluble	0.57 (80.3)	1.02 (53.4)

Isolated protein bodies and vacuoles (~1 mg protein in 100 μ l) were mixed with 1 ml of water. After removing the insoluble protein by centrifugation (10,000g, 10 min), the soluble protein was precipitated with 1 ml of 10% trichloroacetic acid, dissolved in NaOH and assayed by the Lowry procedure⁷. The insoluble protein was calculated by subtracting the amount of soluble protein from the amount of total protein determined on NaOH extracts of trichloroacetic acid precipitates of initial samples. Values in parentheses show percentages.

Seeds of castor bean (*Ricinus communis* cv. Hale) were soaked in running water for 1 d and germinated in moist vermiculite at 30°C. Vacuoles were prepared as described previously², using 140 endosperm halves, removed from 4-d-old seedlings. The protein bodies were prepared from 20 dry seeds by the non-aqueous method of Yatsu and Jacks⁴. Figure 1a, b are photomicrographs of the isolated protein bodies and vacuoles, to the same scale. From analysis of total protein and from haemocytometer counts of appropriate samples, it was determined that the protein content of 10⁶ protein bodies was 0.71 mg and that of 10⁶ vacuoles 1.92 mg. Thus, at 4 d, each isolated vacuole still contains an amount of protein equivalent to several original protein bodies, as anticipated from the microscopic examination³.

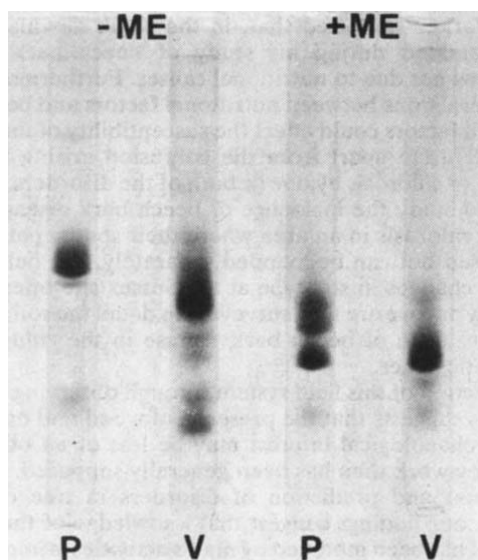


Fig. 2 SDS electrophoretograms of the protein bodies (P) and vacuoles (V) shown in Fig. 1. Samples containing approximately, 70 μ g protein were subjected to SDS-polyacrylamide gel electrophoresis in the presence and absence of β -mercaptoethanol (ME) according to Tully and H.B.⁵.

As shown in Table 1, only 20% of the total protein in protein bodies is water soluble, compared with 50% in the 4-d vacuoles. This indicates that digestion of the (water-insoluble) crystalloid protein is proceeding. Figure 2 shows typical SDS electrophoretograms of protein bodies and vacuoles. These were carried out as described by Tully and H.B. and approximate molecular weights of components estimated⁵. The whole protein body preparation yielded one major band (mw 60,000) and several minor bands, as previously shown⁵. In the vacuole preparation, the major band had disappeared and new protein bands, with lower mws (40,000, 30,000 and 18,000) were present. In the presence of β -mercaptoethanol, three major bands (mws 41,000, 35,000 and 30,000) were seen after electrophoresis of the whole protein body preparation, whereas two

of these (mws 41,000 and 35,000) were not present in the vacuoles. These results show that larger protein components had been converted to smaller polypeptides, as expected from the action of hydrolytic enzymes known to be present in the vacuoles.

The vacuoles contain relatively high amounts of free amino acids (~90 nmol per mg protein). When intact vacuoles, as isolated in 40% sucrose, were incubated at 37°C, the amount of free amino acid increased with time (Fig. 3a), showing that hydrolysis of endogenous protein was sustained in isolation.

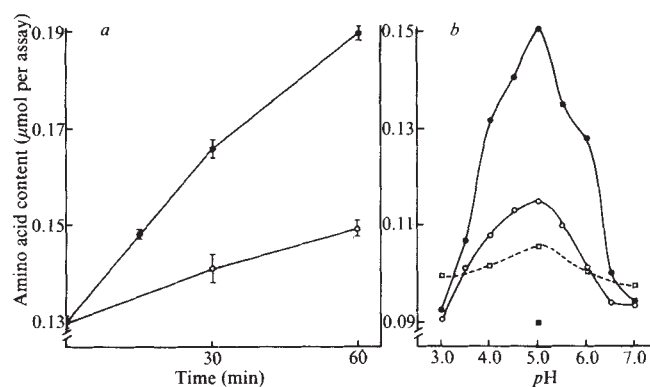


Fig. 3 a, Progress of amino acid accumulation in isolated vacuoles. For intact vacuoles (○), 40 μ l of 50 mM citrate buffer, pH 5.0, in 40% sucrose was added to 10- μ l samples of vacuolar preparation containing 156 μ g protein. To obtain lysed vacuoles (●), 40 μ l citrate buffer, pH 5.0, without sucrose was added to similar aliquots of vacuoles. Samples were incubated at 37°C and the reaction terminated with 50 μ l 10% trichloroacetic acid. After centrifugation the supernatant solutions were analysed for free amino acid using the ninhydrin method⁶, with glycine as standard. b, Aliquots of a vacuolar preparation (105 μ g protein) were treated as above to give intact and lysed vacuoles; the pH of the citrate buffer was varied as shown. Intact vacuoles were incubated for 30 min (□), and lysed vacuoles for 30 min (○) and 60 min (●) before amino acid analysis. A zero time control (■) is also shown.

Much higher autolytic activities were observed when the sucrose concentration of the medium was reduced by a four-fold increment of citrate buffer. As shown in Fig. 3b, the optimum pH for autolysis in both intact and lysed vacuoles was 5.0, although the response of the lysed vacuoles showed a much sharper peak.

Thus, the vacuoles show evidence that hydrolysis of endogenous protein has occurred within them before isolation and that this process is sustained in isolation. This seems to be the first direct demonstration of the proteolytic function of these organelles in higher plants.

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Beech bark disease and archaeological crop marks

THE presence of 'crop marks' in aerial photographs is frequently used to detect archaeological features. These marks are produced by differences in plant size or colour which are related to the characteristics of the soil overlying or comprising the man-made feature. Pure stands of many arable crop species are known to show visible responses to soil heterogeneity. In the case of woody perennials we have found only one report describing the detection of archaeological structures by virtue of their influence on the growth of a crop¹. This was the identification of ancient hut sites in Kenyan tea plantations where excess lime caused failure of the crop. We now report the detection of an ancient field system by virtue of its present-day relationship to patterns of a disorder in beech (*Fagus sylvatica* L.).

An aerial photograph of pure beech stands planted between 1920 and 1948 at Marden Forest, West Sussex, UK, shows some intriguing 'geometrical' patterns of chlorosis. In the area shown in Fig. 1 the trees were 25 yr old when the photograph was taken in 1973. A ground survey of this area revealed a series of scarps up to 1.4 m in height on gently sloping ground. The distribution of these scarps and their structure, as revealed by excavation of two of them, suggests that they are the remains of a field system of prehistoric or Romano-British origin. We have surveyed these scarps or lynchets, and Fig. 2 shows their relationship to the pattern of chlorosis plotted by photogrammetric techniques. Most of the affected trees seem to occur immediately below the scarps.

Our original purpose in examining the photograph had been to detect spatial patterns of beech bark disease which is present at this site and is caused by the scale insect *Cryptococcus fagi* Bär. and the fungus *Nectria coccinea* (Pers. ex Fr.) Fries. Like many plant diseases, beech bark disease has been surveyed using aerial photography⁴, the symptom which makes such detection possible being foliar chlorosis⁵. This symptom may, however, be caused by other disorders; in particular, a nutritional deficiency in which beeches growing on thin calcareous soils are unable to absorb iron in adequate quantities².

The natural occurrence of nutrient imbalances in soil would not normally follow a spatial pattern of the type displayed by the chlorotic trees. At this site the soil is underlain by chalk and, in its undisturbed state, it probably included a shallow capping of slightly acid material. It is generally considered that in field systems of the type found here, gradual erosion over many years



Fig. 1 Aerial photograph of geometrical patterns of foliar chlorosis at Marden Forest. (Crown copyright reserved.)

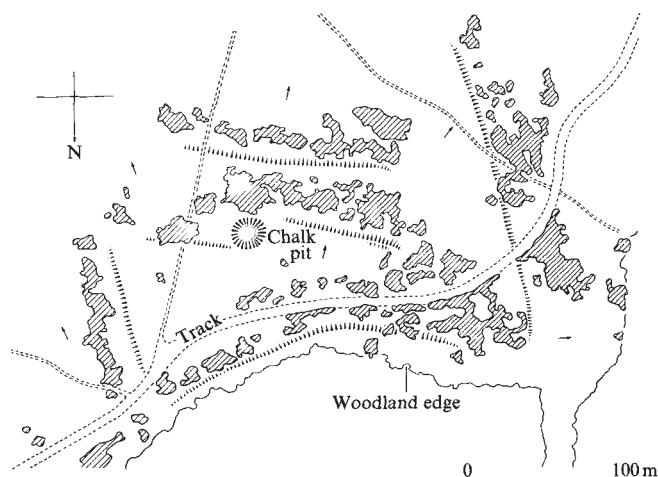


Fig. 2 Map of ancient field system and associated areas of foliar chlorosis. Hachures represent scarps (field boundaries). Cross-hatching represents chlorosis. The arrows indicate the direction of the down-slope.

of ploughing along the contour has removed soil from the areas below the field boundaries. Apparently this process, together with disturbance of the underlying chalk, has produced a soil alkaline to the surface so that conditions conducive to nutritional chlorosis have arisen very locally and in a geometrical pattern.

Aerial detection of beech bark disease in areas where nutritional chlorosis is present would seem to be very difficult, although Parker⁵ reported that, in the chalk downland plantations examined during his study of beech bark disease, chlorosis was not due to nutritional causes. Furthermore, there may be interactions between nutritional factors and beech bark disease. Soil factors could affect the susceptibility of the trees to the disease, quite apart from the confusion arising from the production of chlorosis by one or both of the disorders. It would be useful to study the incidence of beech bark disease and of nutritional chlorosis in an area where their spatial patterns are superimposed but can be mapped separately. We believe that the abrupt changes in soil type at the Sussex site offer such an opportunity and we are now surveying in detail the soil variation and the incidence of beech bark disease in the chlorotic and non-chlorotic zones.

The detection of this field system through observing chlorotic tree crowns suggests that the presence of woodland on a site of possible archaeological interest may be less of an obstacle to aerial survey work than has been generally supposed. As far as the detection and prediction of disorders in tree crops are concerned, our findings suggest that knowledge of the ways in which a soil has been modified by man's activities is important to the plant pathologist as well as to those who are responsible for establishing and managing plantations.

We thank Mr H. C. Bowen, of the Royal Commission on Historical Monuments, for discussions.

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reviews

A revaluation of all values

George Steiner

Science in a Free Society. By P. Feyerabend. Pp. 221. (New Left Books: London, 1978.) £7.50.

FORMALLY, this book is a mess. It is a patchwork of polemic, of autobiographical fragments, of re-iterations of or rapid addenda to an earlier book, *Against Method: Outline of an Anarchistic Theory of Knowledge* (New Left Books: London, 1978). In fact, it will make almost no sense to anyone who has not read Paul Feyerabend's main tract or who has not kept its principal arguments and areas of reference clearly in mind. The tone is, once again, truculent, self-serving, caustically dismissive of dissent and, at pivotal moments, rhetorical. A paragraph, chosen almost at random, will communicate the flavour and purpose (one can choose almost at random precisely because the technique of argument is one of circular incantation):

Paul Feyerabend

"Of course, our well conditioned materialistic contemporaries are liable to burst with excitement over events such as the moonshots, the double helix, non-equilibrium thermodynamics. But let us look at the matter from a different point of view, and it becomes a ridiculous exercise in futility. It needed billions of dollars, thousands of well-trained assistants, years of hard work to enable some inarticulate and rather limited contemporaries to perform a few graceless hops in a place nobody in his right mind would think of visiting—a dried out, airless, hot stone. But mystics, using only their minds travelled across the celestial spheres to God himself whom they viewed in all his splendour receiving strength for continuing their lives and enlightenment for themselves and their fellow men. It is only the illiteracy of the general public and of their stern trainers, the intellectuals, and their amazing lack of imagination that makes them reject such comparisons without further ado."

The sleight-of-hand whereby moonshots, work on DNA and the mathematics and/or technology of non-equilibrium thermodynamics are conflated is no less representative of Professor Feyerabend's method than is the bullying lack of punctuation throughout the latter part of the passage.

As readers of *Against Method* will know, the author has two principal and related aims: to demystify the alleged rationality and methodological integrity of the 'exact' sciences and to remove these sciences from the prestigious, politically and socially determinant rôle which they play in current western society. To borrow Nietzsche's tag, Professor

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Feyerabend is arguing for 'a revaluation of all values':

"Bringing a faint smile to the faces of people who have been hurt, disappointed, depressed, who are paralysed by some 'truth' or by the fear of death seems to me an achievement infinitely more important than the most sublime intellectual discovery: Nestroy, George S. Kaufman, Aristophanes, on my scale of values range far above Kant, Einstein and their anaemic imitators."

Feyerabend's position might be defined as that of a 'therapeutic anarchist' (it is very close to that of an educational iconoclast such as Ilich, who figures in the text if not in the index). The enemy, among others, is Popper: Sir Karl is "a mere propagandist" whose teachings and following constitute "a tiny out-house" (elegance of expression is not among Professor Feyerabend's weaknesses). But the fundamental enemy—and here Feyerabend's polemic is precisely on target—is "the rise of intellectualism in Ancient Greece", and the unfolding of this intellectualism in the methodologies of Descartes and Kant and in the scientific world-image of Newton and his modern inheritors. It is this 'intellectualism', this belief ('illusory, naïve, finally ludicrous, says Feyerabend) in 'objective truths' and in the disinterested passions of abstract, mathematically-grounded theorising, which have brought the west to its current state of confused inhumanity. Trapped in our naïve, wasteful 'scientism', we no longer even have access to the pluralistic possibilities inherent in the

'animism', in the 'mysticism', in the intuitive, mythologically articulate mentalities and wealth of humane insight in cultures, in modes of apprehension which we have ostracised as 'primitive' or 'irrational' (as if the actual proceedings which led to the Copernican revolution or to Einsteinian relativity were not, in their own way, 'primitive' and 'irrational').

The text is most rewarding where it tells of the personal and pedagogic road which led Paul Feyerabend to his current stance. Kierkegaard's anti-rationalism was an incitement. Dada and the expressionist send-ups immediately after World War I were important (Feyerabend is absolutely right when he notes that Dadaists went deeper than anyone else in identifying the creative and 'deconstructive' "miracle of language"). There were Austrian Marxism and the Vienna School, with its ultimately unfulfilled promise of a rigorous yet also accurately descriptive logic of the sciences. And, above all, perhaps, there was California, with its plethora of informalities, instant utopias, 'Orientalisms', 'counter-cultures' and liberating sunshine.

If Feyerabend often knocks down straw-men—who now refuses to argue the status of the exact sciences, what serious mind is not worried about the technocratic enormities in our environment and politics?—he also scores shrewd hits. He is acute on the innocent 'scientism' of the Vienna Circle; his critique of positivistic paradigms amends and deepens Kuhn. He is robustly anti-parochial, following Protagoras in the conviction that a pluralistic view of values and ideals is the only way of stepping beyond one's village towards the human community at large. Thus, Professor Feyerabend's plea for a new catholicity of understanding is often poignant and persuasive. It is also, now and again, great fun. That a more patient sensibility, that a style of inquiry less contemptuous of disagreement, would give to *Against Method* a more effective place in the present climate of discourse, are considerations which, one fears, Paul Feyerabend would regard as time-serving, silly or both. The dust-jacket photo of the faintly sneering author seems to declare as much. This is a pity, for the issues raised are of the first importance. □

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Taken from the dust-jacket

Correlation of morphology with chemical composition

Electron Probe Microanalysis in Biology. Edited by D. A. Erasmus. Pp. 248. (Chapman and Hall: London, 1978.) £15.

OFFERING the capability of chemical analysis *in situ* at a subcellular level, electron-probe X-ray microanalysis is an exceptionally effective means for the correlation of morphology and chemical composition. Although the importance of the technique in biology is now well established, there are few texts giving the newcomer all-around guidance in its biological use. Indeed, aside from the volume under review, only the 1977 book by John Chandler comes to mind.

The editor's introduction is followed by a capable exposition by J. C. Russ of physical fundamentals and limits of performance. The next two chapters are by J. A. Chandler ("The application of X-ray microanalysis in TEM to the study of ultrathin biological specimens—a review") and by A. J. Morgan, T. W. Davies and D. A. Erasmus ("Specimen preparation"). Although there is considerable overlap between these sections, Chandler's chapter features a very extensive coverage of the literature on biological applications, whereas Morgan *et al.* provide a much fuller comparison of a variety of preparative procedures. The chapter by T. C. Appleton ("The contribution of cryo-ultramicrotomy to X-ray microanalysis in biology"), is mainly a good up-to-date presentation of the difficult procedure of preparing ultrathin cryosections of quench-frozen material. In the following chapter ("The application of X-ray microanalysis to histochemistry"), I. D. Bowen and T. A. Ryder give a critical review of precipitation techniques with particular attention to enzyme localisation. The final section, "X-ray analysis applied to the study of renal tubular fluid samples" by H. O. Garland, J. A. Brown and I. W. Henderson, describes the special techniques used for the quantitative analysis of

tiny samples of fluid obtained by micropuncture and documents the effectiveness of the electron-probe method in this field.

Two limitations of the text should be noted. Firstly, as the editor states, "The field is . . . enormous and in order to follow certain aspects in depth, the contents of this book have been restricted essentially to the analysis of specimens in the transmission electron microscope." Nevertheless, although the emphasis on transmission microscopy is plainly evident, much of the contents, especially with regard to specimen preparation, applies equally well to studies based on scanning electron microscopy, and indeed the published X-ray analyses of micropuncture specimens have generally been done with scanning-type instruments.

Secondly, as an over-view, the text is very limited in the area of studies associated with cryotechniques, the

quantitative analysis of diffusible elements, and the attempt to control radiation damage by means of low temperatures. Although Appleton's chapter gives an excellent summary of procedures for preparing cryosections, major results from several laboratories on the quantitative analysis of diffusible elements in cryosections have been published too recently for inclusion.

However, the analysis of diffusible elements in cryosections is a field still in its infancy, and in any case requires specialised facilities. For the biological electron microscopist whose EM column is fitted with X-ray microanalytical facilities, the book gives a good idea of what can be done relatively readily, and how to begin doing it.

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Selective controlled fusions of membranes

Cell Surface Reviews. Vol. 5: Membrane Fusion. Edited by G. Poste and G. L. Nicholson. Pp. 862. (North-Holland: New York and Oxford, 1978.) \$120.

SELECTIVE, controlled fusions of membranes have long been recognised as major features of cellular and subcellular interactions in living organisms but only in recent years has any concerted effort been made to establish their significance and to identify the molecular events involved. A comprehensive treatment both of the phenomena and of attempts to account for them at the molecular level is thus both opportune and welcome.

Poste and Nicholson have made excellent choices of topics and authors to provide this comprehensive coverage, and the individual authors (with one or two exceptions) have poured all they had into it. The result is a rather large book that takes a long time to read, but the articles and their extensive reference lists (with titles) provide a reservoir of information which those directly involved in fusion studies will explore with relish and at which those with more limited interests will nibble with profit.

Most of the book is inevitably taken up with descriptions of the circumstances and conditions in which membrane fusion takes place both *in vivo* and *in vitro*. Fusion events involved in fertilisation in invertebrates (chapter 1) and in vertebrates (chapter 2) are

discussed in largely phenomenological terms. Somatic and sexual fusions in myxomycetes and in fungi (chapter 5) are more amenable to studies of control factors, as also are myoblast fusion (chapter 3) and macrophage fusion (chapter 4). The latter can be studied in culture conditions as well as *in vivo*. Plant cell protoplasts also provide a useful system for the study of the conditions required for cell fusion (chapter 8) now that effective means of removing cell walls have been devised. Fusion events which transfer both vesicle-enclosed material and membrane itself through the intracellular system during endocytosis and/or exocytosis are also extensively covered (chapters 9–12).

Detailed descriptions of chemically induced (chapter 6), virus-induced (chapter 7) and Ca^{2+} -induced (chapter 8) fusions *in vitro*, provide a basis for speculations concerning factors which may contribute to fusion processes *in vivo*. Throughout the book there are repeated references to possible influences of the nature and distribution of lipid components, the nature and distribution of protein and glycoprotein components and levels of Ca^{2+} ions on a variety of fusion processes, and there is a final chapter on problems in the physical interpretation of membrane interactions and fusion.

Thus this volume provides an authoritative, detailed and up-to-date assessment of studies relating to membrane fusion which will contribute substantially both to our understanding of and experimental approach to the problem.

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Erratum

● The review of *A Review of Amino Acid Transport Processes in Animal Cells and Tissues* (Nature, 4 January; 277, 73, 1979) was by C. W. I. Owens, who is Lecturer in Clinical Medicine, and not as stated.

Transform techniques in chemistry

Transform Techniques in Chemistry. Edited by P. R. Griffiths. Pp. 385. (Plenum: New York and London, 1978.) \$47.40.

MANY spectroscopists, particularly those using nuclear magnetic resonance, have had their field revolutionised in the past few years by the introduction of Fourier transform techniques, but have been forced to pick up the new folklore in a haphazard manner for lack of a suitable general treatment of transform methods written from the point of view of the chemist. This excellent new book goes a long way towards satisfying this need. It provides a convincing demonstration that the concept of Fourier transformation is common to a whole range of physical techniques, ranging from the well-known NMR spectroscopy and infra-red interferometry to the more recent Fourier transform ion cyclotron resonance. Although Fourier transformation naturally dominates the discussion, it is shown that related techniques such as the Hadamard and Walsh transforms can also

be useful to the spectroscopist in suitable circumstances.

This book is a collection of chapters by different authors, and can be divided into the mathematical and data handling topics (chapters 2-4, 7, and 11-13) and applications to specific types of spectroscopy or chemistry (chapters 1, 5, 6, 8-10 and 14). This method of compiling a book has the obvious strength of allowing the important sections to be written by the appropriate expert; in the present case there is an excellent description of Fourier transform ion cyclotron resonance by Comisarow. On the other hand, multiple authorship can mean undesirable repetition. Worse still, a given topic may be treated superficially several times over, no single author wishing to devote enough space for a definitive discussion. For example, the practice of 'zero-filling' interferograms raises some fairly fundamental questions that are seldom answered to the satisfaction of the average spectroscopist, and one would have expected the present volume to tackle this hot potato once and for all. Instead the subject is treated separately in chapters 4, 8 and 11, in each case rather uncritically.

This is an extremely useful book for locating those elusive but vital pieces

of information the research worker always seems to be looking for; at the same time the general concepts are very well treated. Most readers will be surprised at the number of different areas where transform techniques are used and how related problems turn up in different forms in the different fields. This broadening of our understanding could well lead to new experiments inspired by the methods used by our colleagues. Everyone will find something new in this book. Even the jargon of our fellow spectroscopists can come as a surprise; the reviewer particularly noticed "dichotomizer", "cross-correlogram", "decimation" and "transgeneration".

In spite of the use of multiple authors, the material presented is remarkably up-to-date, and there seem to be no glaring omissions. It would have been nice, however, to have seen some mention of Fourier transform methods of NMR imaging (zeugmography) and the developing field of two-dimensional Fourier transformation.

Ray Freeman

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Soil profile recording

Soil Sampling and Soil Description. By J. M. Hodgson. Pp.241. (Clarendon Oxford University Press: Oxford, 1978.) £10.

FROM the outset it is essential to realise that this little book is not intended to be a field handbook. Rather it is a review of 'how and why' specific profile features should be recorded together with a summary of the descriptive methods adopted by various national and international organisations.

In the opening chapter the author considers the reasons for studying the soil in the field and the need to describe accurately profile features. Chapters 2 and 3 provide a concise review of methods of profile site selection, description and preparation.

The 'how and why' of soil description is the central theme of chapter 4, which provides a comprehensive list of the soil features the field scientist is likely to encounter. Unfortunately the approach adopted in this chapter is by no means uniform. Each feature is dealt with in turn; in some instances the reasons for selecting a particular feature are discussed in detail, whereas

in others the importance is assumed to be obvious. Similarly when dealing with possible means of measuring or classifying a particular feature the methods are sometimes considered in detail, as for example in the case of structure or as in the case of roots only perfunctorily.

This long account is followed by two succinct chapters covering techniques of data recording and sample collection. Of particular value is the section on recording of data in a form amenable to computer storage. (In this section figures 5.5 and 5.8 are transposed.) The final chapter is in the form of a summary of the profile features used in soil description by some 28 different organisations. The reviews are necessarily brief and details are only given where the procedure or feature used differs from those in the USDA 1951 Handbook. This chapter is valuable if only because it brings together for the first time the approaches adopted by a variety of agencies.

Overall one is left with the question of what is the purpose of this book? Had the chapters on the 'how and why' of soil description considered the question of 'why' in greater depth then this would indeed have been a useful text.

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Quantum chemistry

Quantum Chemistry. (Reference Edition.) By J. P. Lowe. Pp. 599. (Academic: New York, San Francisco and London, 1978.) \$49.50; £32.15.

QUANTUM CHEMISTRY signifies different things to different minds. Some forget the 'chemistry' and provide for chemists simplified, or simply curtailed, versions of books on quantum mechanics. Some concentrate on molecular properties with and without spectroscopy; others concentrate on molecular structure. The last are increasingly based on computer techniques—as they should be—for they have provided the most important quantitative advances in recent years. In them lies the hope of simulating more precisely the processes that occur when molecules undergo the dramatic changes that result in reaction, and in them lies one of the very few tests we possess of the qualitative ideas that underlie our understanding of the equilibrium geometries of molecules as well as their reaction characteristics.

Dr Lowe has written a book that covers parts of this well trodden ground. It is aimed principally at readers whose interest is in molecular structure, and to say what is not in it may be as helpful as saying what is in it: thus it is not a spectroscopy book, nor is it a molecular properties book. It does, however, deal with basic quantum mechanics (but reserves operator properties until well on into the text), atomic structure, and then, for over half the book, molecular structure. The level of treatment of molecules is slightly beyond what undergraduates normally meet, and covers basic LCAO–MO theory, Hückel theory, and SCF–MO theory. The final chapter, which I suspect the author is proud of most, is on 'qualitative molecular orbital theory', which turns out to be the Walsh-type argument for structure and Woodward–Hoffmann rules for reactivity.

Although the basic material is treated in other books with similar titles, there are aspects of the treatment that make the book attractive. The author clearly has an eye for the interesting analogy and felicitous example, and readers will enjoy the sensitivity of his exposition. There are places where he apparently has not troubled to think up an explanation (for example, the Hund rules), but on the whole readers will find themselves in the presence of an imaginative and reliable interpreter.

At various points I thought the author's judgement faltered. For instance, I think very little purpose is served by working through in detail the old-fashioned way of solving the harmonic oscillator Schrödinger equation; and even less is

served by sketching the hydrogen atom solution before talking about angular momentum, leaving angular momentum itself largely unexplained, and being unhelpful about spin. Hückel theory is old hat, but it was nice to see modern judgements (and comparison with experiments, as in hyperfine coupling constants) brought into the discussion. Various nice examples of perturbation theory are presented, and group theory is

presented in a suitably pragmatic way.

In summary, Dr Lowe has written a nice book, which covers old ground in a pedagogically attractive and largely contemporary way. Readers will find it an agreeable, accessible commentary on modern structural quantum chemistry.

P. W. Atkins

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Insect behaviour

Insect Behavior. By R. W. Matthews and J. R. Matthews. Pp. 507. (John Wiley: Chichester, UK, and New York, 1978.) £15.85.

It is rare to find a major gap in the present plethora of scientific books but a treatise on insect behaviour has been badly needed for years. There is surely more published work on this subject than on the behaviour of any other animal group. A great part is descriptive and no small part anecdotal but insect behaviour has also been the subject of innumerable analytical studies, the special strength of which has been their leaning toward physiology rather than psychology.

This book makes a bold and within its purpose successful attempt to fill the gap. Faced with the superabundance of information and the infinite diversity of insect behaviour the authors had to choose between breadth and depth, and chose breadth. Comprehensiveness probably was in fact the most pressing requirement for a first book in the field, one that would serve as a detailed 'guidebook' for entomologists and behaviourists generally.

Inevitably it will be said that in trying to cover everything the authors succeed in saying little about anything. Again and again the reader is tantalised by the opening of an interesting topic only to find it closed again for another. However, while holding to their comprehensive purpose the authors have gone some way to meeting this criticism by frequently interpolating fuller 'case-studies', and by ending every small subsection with citation of up-to-date reviews and books on that specific theme, an invaluable feature. It is difficult to think of any topic that does not get some introductory treatment in the book: slave-making and *Zeitgebers*, cannibalism and coyness, heritability, flicker-fusion, the r - K spectrum, Solanum alkaloids, stimulus filtering, bioluminescence, parental investment . . . it is all there. The large Subject Index unfortunately does not adequately underpin the guidebook func-

tion, the bulk of it being insect names. Let us hope the authors will provide a fuller one in future editions.

The authors describe the general approach of their book as "ethological", meaning a primary concern with descriptions, functions, behavioural ecology and evolution. After an introductory chapter including a warning against anthromorphism and teleology, one chapter of 40 pages deals sketchily with nervous and hormonal mechanisms. The rest of the book is devoted to the gamut of behavioural activities under two main heads, "maintenance activities" of individuals, and "communicatory activities" between individuals of the same or different species. Maintenance is covered in chapters on "Spatial Adjustment" (locomotion, orientation, dispersal and migration) and "Feeding Behavior" (plant relations, food location, social feeding, food recognition and feeding regulation).

Communicatory activities take up the remaining 300 pages out of the total of 500, an emphasis reflecting the enormously increased recent academic and practical interest in these matters. Chemical, visual and mechanical (including acoustic) communication each have a chapter followed by three more strictly focussed on activity types, defensive and reproductive behaviour, brood care and social life. The arrangement is novel and effective. Illustrations are profuse, one on almost every other page, and include many photographs which have not all reproduced well. The reviewer has found one or two erroneous passages and it would be astonishing if there were not more in such an ambitious undertaking. There is naturally a certain transatlantic bias: it is strange, for example, not to find F. Huber's work in an account of acoustic communication.

The book remains a monumental guidebook which insect and other behaviourists will want to have access to for years to come.

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